OBSERVATIONS ON IMMUNE RESPONSES OF SHEEP TO ORF VIRUS.

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DECLARATION

I declare that this thesis has been composed by me.

The wind doth blow,
And we shall have snow,
And what will poor Robbin do then?
He'll sit in a barn,
To keep himself warm,
And hide his head under his wing.

Anon., 1805.

Dedication

To Martha, Phoibe and Kella for their love, patience and encouragement.

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LIST OF ABBREVIATIONS AND ACRONYMS

As	absorbance of the standard serum.
Ax	absorbance of the serum samples.
BB	barbitone buffer.
BCG	bacillus Calmette-Guerin.
C	Celsius.
CFT	complement fixation test.
Ci	a unit of radioactivity defined as a distegration rate of 3.7×10^{10}/S.
CMI	cell-mediated immunity or cell-mediated immune...
cpm	counts per minute.
CT	calf testis.
DE 52	diethylaminoethyl cellulose.
DDW	deionized distilled water.
DNA	deoxyribonucleic acid.
EGM	Eagle$s minimum essential growth medium.
g	acceleration due to gravity.
g	gram
HBSS	Hank 's balanced salt solution.
HBSS-H	Hank 's balanced salt solution with heparin.
H^3Tdr	Tritiated thymidine.
IgA	major immunoglobulin of the external secretions in man and other mammals where it is found as a dimer linked to secretory piece. Also present in the serum as a monomer and in polymerized forms.
IgE	immunoglobulin associated with reagin.
IgG	major immunoglobulin in the serum of mammals divided into IgG1 and IgG2 subclasses in sheep.
IgM	high molecular weight immunoglobulin (19S).
l	litre.

LIF	leucocyte migration inhibition factor.
LMI	leucocyte migration inhibition.
LT	lamb testis.
MI	Migratory Index.
M	mole.
ml	millilitre.
mmol	millimole.
mg	milligram.
nm	nanometre.
NCS	North Carolina solubilizer.
PB	phosphate buffer.
PBS	phosphate buffered-saline.
PE	peritoneal exudate.
pH	logarithmic index of the hydrogen ion concentration.
PHA	phytohaemagglutinin.
POPOP	1,4-Di-2(5-phenyloxazolyl)-benzene.
PPO	2,5-Diphenyloxazole.
PTA	phosphostungstate acid.
PMN	polymorphonuclear leucocytes.
rpm	revolutions per minute.
SI	stimulation index.
sq mm	square millimeter.
Tris-NAOH	Tris9hydroxymethyl) methylamine in sodium hydroxide.
TClD	Tissue culture infective dose.
ul	microlitre.
uCi	microCurie.

ABSTRACT

Sheep infected and re-infected with orf virus responded with lesions which evolved through classical pox stages of papule, vesicle, pustule and scab. In susceptible sheep they resolved within five weeks whereas in re-infected sheep there was an accelerated response, the lesions healing within three weeks.

Experiments were designed to correlate the roles of humoral and cell-mediated immune responses with clinical responses. Sera were analysed for changes in the levels of total proteins, serum fractions, IgM, IgG1, IgG2 and antibodies. Leucuocytes from infected and re-infected sheep were also assayed for cell-mediated immune responses.

Changes in the total serum proteins of infected and re-infected sheep, pre-challenge and post-challenge were not significant. Contrarily, there were significant increases in the gammaglobulin levels in re-infected sheep and slight increases in the levels in infected susceptible sheep.

IgM levels of infected and re-infected sheep were not markedly altered. IgG1 and IgG2, in contrast, increased significantly after infection and re-infection. The increases correlated positively with orf antibody titres.

The rate of antibody production was significantly slower in infected sheep than in re-infected sheep and the titres attained were much lower in susceptible sheep than in re-infected sheep.

Transfer of sensitized lymphocytes from recovering re-infected sheep to susceptible lambs induced accelerated clinical reactions when these lambs were challenged with orf virus.

A preliminary study of the effects of immunosuppression on the pathogenesis of orf, indicated that corticosteroid delayed the appearances and prolonged the durations of the lesions. It also delayed the onset of antibody production and depressed antibody titres, markedly so in re-infected sheep. It had no effect on the cell-mediated immune responses in infected susceptible sheep.

In conclusion, dynamic relationships were found between the lesion development and the humoral and cell-mediated immune responses in sheep infected and re-infected with orf virus.

CHAPTER ONE

INTRODUCTION

Orf is a benign cutaneous pox disease of sheep and goats. Many terms have been used to describe the condition but the two most common and appropriate are contagious pustular dermatitis and contagious ecthyma.

Although 'solid' immunity was believed to develop after natural infection or vaccination, some antithetical views have recently been expressed. Firstly, most recovered or vaccinated sheep react to secondary challenge with an accelerated response; normally in a primary reaction, the lesions resolve 28 to 40 days after infection but in secondary reactions the lesions take only 10 to 14 days to resolve. Secondly, attempts to passively transfer resistance to susceptible sheep using serum from hyperimmunized or recovered sheep have always failed. Furthermore, lambs from immune ewes, though receiving colostral antibodies, succumb to the virus with a severe primary reaction soon after birth. Moreover, vaccination has been practised for many years and it seems to have had very little effect on the endemicity of orf.

Several authors have suggested the involvement of cell-mediated immune responses in pox viruses infections and that these responses are mainly responsible for the accelerated reactions after re-infections. The lesions associated with re-infections with orf virus, however, are unlikely to be due to a delayed-type hypersensitivity because Osman (1976) detected virus multiplication in the lesions. He also observed rapid clearance of the virus

and the purpose of this investigation is to establish the possible explanation of this phenomenon. The present project, was therefore, aimed at ascertaining to what extent humoral and cell-mediated immune responses were correlated with primary and secondary reactions to orf virus.

CHAPTER TWO

REVIEW OF THE LITERATURE

PATHOGRAPHY OF ORF

General

Orf was recognised as a contagious disease of sheep and goats in Europe as early as the middle of the eighteenth century (Steed 1787). It is a benign disease characterised by the development of nodular lesions at any site where the integument is broken. The commonest sites, therefore, are the lips particularly the commissures, muzzle, nostrils, around the eyes, coronets and the teats. Lesions will also appear at any other site with a wound. The aetiological agent is a parapoxvirus (Fenner and White, 1976).

Epidemiology

The disease has a world-wide distribution and occurs wherever sheep and goat husbandry is practised. At present, according to the FAO-WHO-OIE Animal Health Yearbook (1977), the disease is of economic importance in Europe, Australia, New Zealand, South Africa, Nigeria, Zaire and India.

The natural hosts are sheep and goats of any age. Orf has also been diagnosed in wild thar (Kater and Hansen 1962), chamois (Grausgruber 1964), alpacas (More 1965) Musk ox (Kummeneje and Krogsrud 1978) and reindeer (Kummeneje and Krogsrud 1979). Man is suceptible, being infected when handling infected animals (Hart, Hayston and Keast 1949; Nagington and Whittle 1961).

Similarly, dogs which had eaten an infected sheep skin became infected (Wilkinson, Rydie and Scarnell 1970).

Walley (1888) described orf as a pox-like infection but he believed that the causative agent was Fusiformis necrophorus, an opportune bacterium which was often isolated from sheep naturally infected with orf. In 1923, Aynaud demonstrated a filtrable virus which survived in dry scabs and caused a disease with some similarities to those caused by vaccinia-variola viruses.

Aetiology

The development of electron microscopy enabled Nisbett (1954) and later Nagington, Newton and Horne (1964) and Peters, Mueller and Buttner (1964) to study in detail the morphology of orf virus and its relation to other pox viruses. They described orf virus as a monomorphic short rod with rounded ends, approximately 252 nm by 158 nm in size. A tubular thread of 8 to 10μ and 7 to 9 nm diameter was woven around the core giving the whole virus particle a ball of yarn appearance. The morphology and size of the orf virion closely resembled that of other pox viruses. The dry weight of the whole virion was 3.69×10^{-15} and the molecular weight was 1.71×10^{-6} (Ichihashi, Matsumoto and Dales 1971).

Peters and his colleagues (1964) showed that milker's nodule virus, orf virus and bovine papular virus (BPS) were morphologically more closely related to each other

than to the vaccinia-variola poxviruses. They therefore, suggested that they comprise a new group for which they proposed the name, the paravaccinia of the poxviridae. Nagington, Tee and Smith (1965) further proposed that the group be renamed the orf sub-group because orf virus was the first in the group to be described in detail. Earlier, in 1958, Webster had found immunological relationships among orf, vaccinia and ectromelia viruses using complement fixation and double diffusion tests. Later, Woodroofe and Fenner (1962) showed that soluble antigens from pox viruses were sub-group specific and nucleoprotein antigens were group specific. This was confirmed by Papadopoulos, Dawson, Huck and Stuart (1968).

Several workers have shown that there is no plurality of orf virus and that the viruses isolated from different localities and designated as 'strains' in fact all conform to a single type (Glover 1933; Manley 1934; Horgan and Haseeb 1947; and Olah and Elek 1952).

Cultivation

Orf virus was first cultivated successfully in 1957 when Greig established virus obtained from infective scabs in cell monolayer culture derived from embryonic sheep skin cells. Subsequently, the virus has been established in other cell culture systems using both primary cell cultures and cell-lines. Plowright, Witcomb and Ferris (1959) for example reported that orf virus from in-

fected lambs caused cytopathic effects in cell monolayers derived from lamb and calf testis and kidney, and MacDonald and Bell (1961) managed to grow the virus in cell cultures from embryonic human liver cells. The cytopathic changes in the cell cultures infected with orf virus as reported by Greig (1957), Plowright and his colleagues (1959) and Osman (1976) included discrete refractile cells, round giant cells, large paranuclear cytoplasmic inclusions of ill-defined area, and varying degenerative changes in the nuclei of infected cells.

Nagington (1968) studying the behaviour of paravaccinia viruses in cell culture derived from different host species, concluded that the main difference in their behaviour occurred during the initial isolation from infective material. Orf virus from sheep and goat lesions grew only in cell cultures from sheep tissues but lost this specificity after a few passages, whereas, BPS virus and pseudocowpox virus on first isolation were non-specific.

Pathogenesis

Experimental infections of sheep with orf virus progress through the classic stages of a pox infection, namely, macules, papules, vesicles, pustules and scabs (Walley 1888; Romero-Mercado 1969; Osman 1976). The evolution of the natural disease is less well-defined; most skin lesions being recognized as encrusted nodules and oral lesions as strawberry-like proliferations.

Uncomplicated lesions arise when the virus gains

entry to the host through broken skin at the site of a small wound or an abrasion and penetrates the epidermal cells. Replication of the virus particles causes ballooning degeneration, nuclear shrinkage, and karyolysis of the cells of the malpighian layers. Cells in the basal layer undergo proliferative activity leading to marked acanthosis and elongation of the retia pegs. Inflammation sets in accompanied by hyperaemia, oedema and accumulation of mononuclear cells, especially in the dermis (Nisbett 1954; Kluge, Cheville and Perry 1972). Healing usually takes place within 40 days depending on the site involved and the age of the animal. Secondary infection caused by bacteria or invasion by parasites such as screw-worm fly larvae can lead to complications which adversely affect healing and result in mortality (Blanc 1922; Howarth 1929; Boughton and Hardy 1934; Hart et al., 1949).

IMMUNOGENESIS OF ORF

General

Several groups of workers have reported that an animal which has recovered from a natural infection with orf virus or which has been vaccinated against orf acquires a 'solid' immunity but there is a considerable difference of opinion as to the nature, duration and degree of this immunity. Aynaud (1923) said that immunity

occurred after the local lesion had healed. Lanfranchi (1925) claimed that immunity followed both natural and experimental disease, but that the immunity was not always of long duration and might disappear after five to eight months. Glover (1928) showed that, under experimental conditions, recovered animals possessed a high degree of immunity which protected them against re-infection for a period of at least eight months. Carrè (1932) reported that animals recovering from natural infection remained immune for two and a half years, whereas, vaccinated animals became susceptible after only one year.

In contrast, Boughton and Hardy (1934), investigating whether vaccination offered a practical and dependable means of protecting lambs from orf, found that not all vaccinated animals were completely immune to challenge when the virus was rubbed into scarified skin. There was a reaction to the second exposure but the reaction was of a milder character and of shorter course. The finding that most of the recovered animals reacted to secondary challenge was confirmed by Hart and his co-workers (1949), Wheeler and Cawley (1956) Schmidt (1967) and Osman (1976). The secondary reaction produced the classical stages of a pox infection but the course and severity of the lesions were markedly shortened compared to the primary reactions. In addition, Osman (1976) found that virus multiplication occurred at the site of re-

infection. Using electron microscopy, Osman (1976) established that the replication of the virus in primary and secondary infections was readily divisible into phases of eclipse, exponential increase, plateau, exponential decline and resolution. The growth curves however, differed dramatically; during the exponential decline phase in the secondary responses, the virus disappeared abruptly whereas, in primary response there was a gradual disappearance of the virus.

Humoral Immunity

The capacity of orf virus to stimulate the production of specific antibodies in the host has been demonstrated by many workers including Glover (1933), Nisbett (1954), Romero-Mercado (1969) and Lutu (1971). Furthermore, Romero-Mercado (1969) managed to differentiate between heat-stable soluble complement-fixing orf antigens and heat-labile soluble precipitating orf antigens. Hence, antibodies in the sera of recovered or vaccinated animals have been revealed using a number of different serological tests.

Complement fixation tests: Glover (1933) using the complement fixation test (CFT) demonstrated the presence of orf antibodies in hyperimmunized lambs but had difficulty in detecting these antibodies in the serum samples from convalescent animals. Similarly, Manley (1934) failed to observe any specific fixation of complement with serum from convalescent sheep but Abdussalam (1958)

demonstrated orf antibodies in sera from both convalescent and hyperimmunized sheep. MacDonald and Bell (1961) using CFT found that orf antibodies developed prior to any other antibodies in experimentally infected sheep. Romero-Mercado (1969) and Lutu (1971) found that in primary infections of sheep with orf virus antibodies capable of fixing complement developed towards the end of the first week after infection and persisted for up to the twentieth week but, when these sheep were re-infected, high antibody titres were not detected by CFT.

Precipitation tests: Orf antibodies detectable by tube precipitation tests were not demonstrated in the sera from hyperimmunized lambs or convalescent sheep by Glover (1933) or Manley (1934). Abdussalam (1958) on the other hand, using tube precipitation tests found that sera from convalescent lambs gave weak precipitin reactions with first supernatant or buffer solution in which orf virus had been suspended and concluded that because of the weak reaction shown by the sera from convalescent animals and none by scab extracts the test was not of diagnostic value for orf as it was for vaccinia. However, Romero-Mercado (1969) and Lutu (1971), using gel diffusion technique, were able to detect antibodies in the sera from naturally and experimentally infected sheep and convalescent sheep by the precipitation tests. They both found that the gel diffusion test was less sensitive for de-

tecting antibodies in sheep with primary reactions to orf virus whereas, high orf antibody titres were readily detected by gel diffusion in sheep re-infected with orf virus or re-vaccinated against orf.

Neutralization tests: Aynaud (1923) was unable to demonstrate any inactivating effect of sera from convalescent sheep on orf virus in a crude scab suspension whereas, Glover (1933) showed that sera from hyperimmunized and lambs/to a lesser extent, sera from convalescent sheep had a neutralizing effect on orf virus suspensions. Abdussalam (1958) verified Glover's findings but MacDonald and Bell (1961) failed to neutralize the effect of the virus using sera from convalescent sheep and infected human beings. Trueblood, Chow and Griner (1963) similarly, failed to inactivate orf virus with serum from hyperimmunized or recovered animals and this led them to suggest that there was lack of protective antibodies in animals infected with orf virus. In addition, Khanduev, Guesev and Dzhakulov (1969) reported that serum from recovered sheep failed to inhibit the growth of orf virus in cell cultures although the sheep themselves resisted re-infection. They, therefore, postulated that protective antibodies might be produced at the site of infection. Poulain, Gourreau and Dautigny (1972) claimed that orf virus adapted to grow in sheep foetal muscle cell culture was neutralized by sera from sheep infected with orf virus, convalescent sheep and sheep vaccinated against

orf. They also claimed that the neutralizing effect was transmitted through the colostrum and therefore, advocated the immunization of pregnant ewes in order to protect their offspring. Boughton and Hardy, however, many years previously (1934) had conclusively shown that colostrum from recently vaccinated ewes did not protect their lambs.

Agglutination tests: None of the many investigators studying the immunology of orf were able to detect orf antibodies in sera from convalescent or hyperimmunized sheep by agglutination or haemagglutination tests (see for example, Glover 1933; Manley 1934; Boughton and Hardy 1934; Abdussalam 1958; Romero-Mercado 1969; Lutu 1971).

Passively Acquired Immunity

Boughton and Hardy (1934) found that the progeny of ewes which were immune to orf did not acquire immunity passively by ingesting colostrum. When such lambs were challenged within 72 hours of birth they developed a severe form of the disease. This observation was corroborated by Trueblood and his colleagues (1963), Romero-Mercado 1969, Lutu 1971 and Kerry and Powell (1971). Kerry and Powell (1971) in addition, found that neonatal lambs responded satisfactorily to vaccination.

Khanduev and his comrades (1969) and Osman (1976) likewise, were unable to protect susceptible sheep from

orf infection by injecting them with sera from hyper-immunized sheep or convalescent sheep.

Cellular Immunity

Boulter (1969), reviewing the situation of protection against poxviruses noted that, although there was general agreement that humoral factors played an important role in acquired immunity to pox virus infection, recent evidence showed that immunity to these infections were not due solely to antibodies. He suggested that the integrity of the cellular mechanisms of immunity, which were manifested by delayed hypersensitivity, were of vital importance in the immunity to poxviruses. Blanden (1971) evaluated the contributions to host responses in the recovery from mousepox infection in susceptible mice in which some were passive recipients of spleen cells from immune mice, some were injected with hyperimmune sera and others received interferon. He found that immune spleen cells transferred highly efficient anti-viral activity into susceptible mice whereas, hyperimmune serum was significantly less virucidal and the interferon was ineffective. He also showed that the immune spleen cells released mediators of cell-mediated immunity *in vitro* when exposed to mousepox antigens.

Although it was suggested long ago that cell-mediated immunity contributed to primary vaccinia virus infections (Portier and Richet 1901; Pirquet 1907) and

that cell-mediated immune responses were responsible for the accelerated reaction to re-vaccination with vaccinia virus against smallpox (MacKinnon and Defries 1931; Craigle and Wishet 1933; Broom 1947), it is only recently that these suggestions have been accepted. A number of clinical conditions have been recognized in human beings in recent years which exemplifies the importance of cell-mediated immune responses in vaccinia virus infections. For example, Glasgow (1970) cited a case of progressive vaccinia in a patient who had no demonstrable abnormalities of humoral immune responses. After immunoglobulin therapy failed to arrest the progress of the lesion, transfer of leucocytes from an immune donor was tried and resulted in improvement and eventual recovery from the infection.

The possibility that immunity to orf might be due to factors other than humoral responses was first suggested by Aynaud (1923) who, having failed to demonstrate any neutralizing effect in serum from convalescent sheep, postulated that immunity to orf was mediated by cellular elements. This hypothesis was not seriously considered until 1976, when Osman attempted to transfer orf immunity to susceptible lambs using immune cells from sheep which had just recovered from orf. He harvested spleen cells from the recently recovered sheep and transferred them into susceptible lambs which he then challenged with orf virus. The results were not conclusive. Nevertheless,

Osman (1976) speculated that the presence of antibodies in the immune sheep played an important part in the accelerated secondary response of enhancing the multiplication of the virus at the site of re-infection.

Enhanced virus virulence has been attributed to maternally derived antibodies in infants and calves infected with respiratory syncytial virus (Kapikian et al., 1969; Kim et al., 1970; Chanock et al., 1970; Smith et al., 1975; MacIntosh et al., 1978). The development of lymphocytic choriomeningitis disease in mice has also been attributed to cell-mediated responses directed against their own virus-infected tissue, and humoral antibodies are thought to modulate this interaction (Rowe 1954; Hotchin 1971). Rowe (1954) had observed that the severity of the disease was reduced in thymectomized neonatal mice but the severity was restored when the thymectomized mice were implanted with thymus cells. However, Osman (1976) did not observe accelerated responses when susceptible sheep were challenged immediately after receiving hyperimmune serum. In fact, typical primary reactions resulted.

IMMUNIZATION

After the aetiology of orf was established numerous workers investigated methods of actively immunizing animals. Aynaud (1923) treated dried infective scabs

with chloroform and glycerine and claimed to have used it successfully as a vaccine. In England, Glover (1933) likewise, claimed to have used successfully a vaccine composed of infective scab suspension in glycerine. Boughton and Hardy (1934) developed a vaccine made out of dry powdered crusts which was diluted immediately before use with distilled water and this was widely used by farmers in the U.S.A. Hart and his colleagues (1949) used suspensions of scabs from naturally occurring cases ground in 50 percent glycerine and saline as a vaccine in Australia. A major hazard of using live virus vaccines was the establishment of foci of infection; vaccination of clean flock therefore was not advised. (Anon., 1973).

Olah and Elek (1953) were able to protect susceptible sheep against orf using formalin inactivated virus. Similarly, Richter and Jansen (1968) found that heat inactivated vaccines evoked a reasonable immunity and recommended this vaccine for flocks not yet infected with orf virus.

Vaccination was recommended just before the risk periods of the year and if the periods were prolonged a six-monthly revaccination was advised (Anon., 1973). Kerry and Powell (1971) advocated the vaccination of a day-old lamb because there was no evidence of maternally acquired immunity in the lambs.

Several commercial vaccines are at present available and contain mild live virus levigated in either glycerine or blue-green base. Wellcome Veterinary Division supply an orf vaccine which consists of glycerinated suspension of scab material obtained from sheep infected with a modified strain of orf virus. Tasman Vaccine Laboratory also supply a mild living orf virus vaccine suspended in a blue-green base recommended for use in sheep prior to the time of the year when the disease usually appears.

Most literature on orf vaccination is open to censure because details regarding methods of assessing the induced resistance are seldom given. There is no quantitative evidence, moreover, that vaccination has altered the epidermiology of orf, other than to ensure its endeminicity.

CHAPTER THREE

GENERAL MATERIALS

AND

METHODS

INTRODUCTION

In this chapter, the general materials, equipment and techniques used throughout the undertaking of the project are described in detail. The formulation of buffers, stains and fixatives and commercial suppliers of reagents are given in Appendix II.

THE VIRUS

The strain of orf virus used was collected from naturally infected sheep on Easter Bush Farm, Roslin, Scotland. The scabs were removed from the lesions using sterile forceps and stored in plastic bijoux at -20°C for periods between six and 48 months. To prepare a 20 percent suspension of the virus particles, the scabs were thawed and then soaked for 15 minutes in phosphate buffered saline (PBS) at pH 7.3 before being ground in a mortar. The PBS diluent also contained 50 mg of streptomycin and 100 units of penicillin per ml. The suspension was centrifuged in glass universal bottles at 1610 g^1 for 30 minutes. The supernatant fluid was harvested and five ml aliquots were stored in glass bijoux at -114°C for use as a source of virus for experimentally infecting animals and inoculating cell cultures.

ANIMALS

Sheep

Suffolk and Cheviot sheep and their crosses were

1 3,000 rpm in MSE Superminor Centrifuge.

obtained from Easter Bush Farm and the Moredun Research Institute, Edinburgh. Most were weaners but maturer and younger lambs were used in some experiments.

The sheep were designated 'susceptible' or 'previously infected' sheep. Those designated as susceptible came from a flock which had been examined clinically every fortnight for more than 10 years and the animals had no apparent signs or history of orf. Those designated as previously infected were animals in which the disease had run its full course after natural or experimental infection with orf.

The animals were housed and fed hay supplemented with a concentrated diet based on oats. During the course of experimental infection they were held individually in metal crates.

Rabbits

New Zealand White rabbits kept individually in cages were used for producing antisera to orf virus, whole sheep serum, sheep IgG1, sheep IgM and sheep IgA. They were fed commercially prepared balanced diets[2].

Goats

White goats were used for producing antisera to sheep IgG2. These goats were housed with sheep and fed hay supplemented with concentrates.

2 Oxoid Ltd.

Guinea pigs

Mature guinea pigs were used for producing peritoneal exudate cells as a source of macrophages for macrophage migration inhibition tests.

EXPERIMENTAL INFECTIONS

Scarification

The first step in infecting sheep was to scarify the skin to permit entry of the virus. A multi-cross pattern was scratched on the inner side of one of the thighs using four sterile disposable blood lancets[1] bound together with a tape. Inoculation of the orf virus was then accomplished by dropping 0.1 ml of 20 percent scab virus suspension onto the scarified skin from a one ml tuberculin syringe and then rubbing the suspension into the wound using the shaft of the hypodermic needle. The animals were examined daily for lesion development using an optical flashlight[2].

There was no obvious systemic response following the application of orf virus suspension to the scarified areas. The local reaction which occurred evolved through the following stages. There was reddening and slight oedema along the lines of scarification on the second and third day after infection. This was followed by development

1 Microlance, Becton, Dickinson and Company.

2 Magnalite, Manning Holoff Company.

of papules then vesicles which contained clear serous fluid on the fourth and fifth day post-inoculation. On the sixth day the vesicles were prominent, raised and confluent. Pustules became manifested on the seventh day. The infected area was at this stage surrounded by a zone of congestion. By the tenth day crusts were evident and had started to dry up along the edges of the lesion to form a firm and brittle scab. Removal of the scab at this stage revealed a raised, raw, bleeding surface. Complete resolution varied; with susceptible animals it took four to six weeks and with re-infected sheep it took two to three weeks..

CELL CULTURE TECHNIQUES

Primary Cell Cultures

Lamb testis (LT) cells and calf testis (CT) cells were employed for primary cell cultures. They were prepared as follows by the method described by Osman (1976) with minor modifications. Fresh testes, removed aseptically from the donor, were placed in a sterile beaker with sterile Hank's balanced salt solution (HBSS) containing 50 mg of streptomycin and 100 units of penicillin per ml. and 10 percent of foetal calf serum[1]. The parenchyma of the testis was exposed, detached from the Tunica albuginea and rinsed several times in HBSS, before

1 Gibco Biocult Ltd.

cutting into small fragments with scissors. The fragments were washed further in HBSS and placed in a conical flask to which four volumes of 0.25 percent trypsin were added to one volume of the fragments. The mixture was gently agitated with a magnetic stirrer for 30 minutes at 37°C. The supernatant fluid was discarded and fresh trypsin added to restore the original volume; trypsinization was allowed to proceed at 37°C with agitation as before. The supernatant fluid was harvested periodically and fresh trypsin added until all the fragments were completely digested. The cells were then sedimented at 4°C, washed twice in HBSS and once in Eagles' Growth Medium (EGM) consisting of 10 percent Eagles' medium in Earles salts[2], 10 percent of new born calf serum[1], 10 percent of tryptose broth, 200mmol of glutamine, 100 units of penicillin, 50 mg of streptomycin and 2.5 ml of 4.4 percent of sodium bicarbonate. The viability of the cells was checked and viable cells counted using the Nigrosin dye exclusion method (Appendix II). The cells were then diluted in the EGM to 10^4 cells/ml and either seeded in test-tubes or Roux bottles or stored in glass bijoux at -114°C after the addition of dimethyl sulphoxide to give a concentration of 7.5 percent.

Established Cell Lines

VERO cells, a continuous cell line originally derived

1 Gibco Biocult Ltd.

2 Wellcome Reagent Ltd.

from African green monkey kidneys, was obtained from a commercial source[1]. They were seeded and maintained in Tissue Culture 199[2] (TC199) growth medium in test tubes or tissue culture flasks.

Adaptation of Orf Virus to Cell Cultures

The virus was grown in primary monolayer of lamb testis cells inoculating 0.1 ml of a 10 percent virus suspension into each tube. On first passage, complete cell destruction was observed within 48 hours. This was also observed in the second passage but in the third passage, cytopathic changes started appearing on the third day post-inoculation in the forms of discrete refractile cells, followed by swelling and rounding up of the infected cells. On the fifth day after inoculation about 50 percent of the cells were clumped or detached from the wall of the culture tubes. The titre of virus obtained was 2.15×10^3 $TC1D_{50}$ /ml in the LT cells and it did not increase despite serial passages.

After at least five passages in LT cells, the virus was further passaged in CT cells to obtain high yields of the virus. The viral titre attained was 3.16×10^6 $TC1D_{50}$/ml.

VERO cell-adapted orf virus was gifted by Dr. Omar A. H. Osman and this was used as source of antigens for serological tests after three passages in VERO cells.

1. Gibco Biocult Ltd.
2. Wellcome Reagents Ltd.

The virus was always harvested from infected cells by immersing the test tubes or culture flasks alternatively in dry ice and warm water three times. The frozen and thawed culture fluid was then centrifuged at 1,600g for 15 minutes. The supernatant fluid was collected and stored in glass or plastic universal bottles at - 20°C.

Virus Titrations

Cell monolayers of either testis cells or CT cells were established on test-tube walls or flying cover-slips in test-tubes and 0.1 ml of a series of ten-fold dilution were added to each tube. Four tubes were used for each dilution. The cultures were then incubated at 37°C for one hour to allow the virus to be adsorbed and penetrate the cells. Maintenance medium containing 5 percent of calf serum was added and the cultures incubated at 37°C. They were examined microscopically every day over a period of seven days for evidence of cytopathic changes. The titres were calculated using the Reed-Muench formula (Lennette and Schmidt, 1969).

The flying cover-slips were stained with Giemsa[1] after fixing the monolayers in methanol and were examined under oil-immersion for intracellular changes.

MICROSCOPIC STUDIES

Light Microscopy

Cell cultures were examined by light microscopy to

1 Giemsa stain.

detect the presence of the virus by the cytopathic changes in the cells. An inverted light microscope[2] was used for examining the cell cultures for confluent monolayers in control uninfected cultures and cytopathic effects in the infected cultures. Stained coverslips of infected cells were examined with a standard light microscope[3] for evidence of cytoplasmic and nuclear changes.

Electron Microscopy

Electron microscopy was the ultimate tool used for confirming the presence of orf virus in scabs, in infected cell cultures and in medium from infected cell cultures.

Direct negative staining was carried out on a copper grid. A copper grid of 400 mesh and 3 mm diameter, coated with carbon was laid on the material to be examined for 30 seconds. The excess fluid on the grid was removed with filter paper. The virus particles were then stained using two percent phosphotungstate acid (PTA) or methylamine tungstate (MT). Staining was done by layering the grid on either PTA or MT for 30 seconds. After blotting dry, the grid was mounted and examined in the electron microscope[4]. *

Ultrathin sections of infected cells were prepared from monolayers of infected cells. Cell monolayers

2 Nikon Model 182813 x 4 objective.

3 Nikon Model 182813 x 100 objective

4 Siemes E.M.

* When present typical orf particles resembling a ball of yarn with strands running diagonally across the particle were observed (Plate I).

infected with orf virus and not yet detached from the glass wall were washed with phosphate buffer at pH 7.4 then fixed with 3 percent glutaldehyde in phosphate buffer (PB) for five minutes, washed three times in PB and then stained with one percent osmium tetroxide for 2.5 minutes. The cells were detached with a glass rod, put in gelatin vials and dehydrated in 30 percent alcohol for five minutes then in 50 percent alcohol for five minutes, 80 percent alcohol for five minutes, 100 percent alcohol for five minutes and finally in epoxy propane for five minutes. The cells were embedded in araldite, sectioned[1] and stained in PTA by placing the sections on the grids.

STATISTICAL ANALYSES

The sizes of the experimental groups were planned for ease of statistical analyses by conventional methods. The commonest techniques used were Student t-tests, Analyses of Variance, Correlation and Linear Regressions.

The significance of changes in total serum proteins, specific serum protein levels, immunoglobulin values, and orf antibody titres was tested by dividing mean differences

1. Huxley microtome.

between the prechallenge and post-challenge values by the standard error of the difference. The significance of its calculated value was ascertained from tables of t-values (Snedecor and Cochrane 1974).

The daily group means of the humoral and cell-mediated immune study groups were compared by the non-paired t-test (Snedecor and Cochrane 1974).

When three or more groups were compared analyses of variance were used. If the comparison revealed significant differences between the means, the data were further analysed by a modified Duncan's multiple-range test to identify the significant subsets (Harter, 1960).

PLATE I: Electron micrograph of orf virus particles.

x 160,000

CHAPTER FOUR

STUDIES OF THE HUMORAL IMMUNE RESPONSES

INTRODUCTION

Immune responses to most antigens are characterised by the appearance of antibodies in the blood or tissues which combine specifically with the antigens that stimulated their production. This antigen-antibody interaction has a number of consequences; for example, the antigen molecules or particles may be agglutinated or precipitated and their effect neutralized or the specific antibody on combining with the antigen may facilitate its uptake and subsequent digestion by phagocytes. In addition, cellular antigens may combine with antibodies and undergo cytolysis by the activation of the complement pathway.

When serum proteins are separated electrophoretically, they separate into alpha$_1$- alpha$_2$-, beta-, and gamma-globulins and albumin. Antibody activity is located mainly in the gamma-globulin fraction and some in the alpha- and beta-globulins. However, with the recognition that there is considerable heterogeneity among the molecules which function as antibodies, they are now generally described as immunoglobulins. Four major classes of immunoglobulins designated IgG, IgM, IgA and IgE have been identified in sheep serum and external secretions and have been characterized by their physiochemical and immunochemical properties, and their metabolic and function features (Aalund, Osebold and Murphy, 1965; Hammer, Kickhofen and Schmidt, 1971; Wells and Eyre 1972; Butler and Maxwell 1972). The IgG class has been further subdivided into two subclasses, IgG1 and IgG2 (Aalund *et al.*,

1965).

Following natural infection with viruses or immunization with a variety of viral antigens the three major classes of immune globulins in the serum participate in the immune response in a sequential manner (Uhr and Finkelstein 1967). The initial antibody response is characterised by the development of IgM which is subsequently replaced by IgG and to a small extent by IgA (Ogra, Karzon, Righthand and Macgillivray 1968). IgG antibody has been found to be more efficient in viral neutralization than IgM antibody but less efficient than IgM in complement fixation and agglutination reactions (Ogra, Morag and Tiku, 1975). The role of IgA is not fully understood, but appears to be one of limiting colonization with a virus at mucosal surfaces since it is the major class of immunoglobulin found in the secretions bathing external mucosal surfaces (Duncan, Wilkie, Hiestand, Winter 1972; Smith, Dawson, Wells and Burrells, 1975, 1976).

Although complement-fixing antibodies and precipitating antibodies are present in sheep which have recovered from orf or in animals which have been vaccinated against orf (Glover 1933; Romero-Mercado 1969; Lutu 1971), the contribution of individual immunoglobulin classes or subclasses has not yet been determined. The purpose of the following experiments was therefore to attempt to characterize the humoral antibody responses to orf virus in sheep infected experimentally.

MATERIALS AND METHODS

Experimental Design

A series of challenge experiments was carried out to follow the development of humoral immunity to orf virus in normal sheep after primary infection, and in sheep re-exposed to orf virus. The animals for the experiments were divided into the following groups:-

Group 1 consisted of 16 previously infected sheep that were challenged with orf virus by scarification.

Group 2 consisted of five previously infected sheep that were not re-infected and were used as uninfected control group.

Group 3 consisted of eight susceptible sheep which were infected with orf virus. This group was set up to show changes occurring in primary orf infections as opposed to the changes in re-infected sheep (group 1).

Collection of Serum Samples

Ten ml of blood for serum samples were collected by jugular venepuncture using "Vacutainers"[1], one day before challenge, then daily for the first two weeks after challenge and thereafter, once every week for a further three weeks. The blood was allowed to clot at 37°C for one hour, centrifuged at 716g for 15 minutes to retract the blood clot. The serum was harvested into glass or plastic bijoux and stored at -20°C until all the samples for the 35 days of the experiment were collected.

1 Becton, Dickinson and Company.

Electrophoresis of Serum Proteins

Changes in the pattern of proteins in the sera of the experimentally infected sheep were examined by electrophoresis on "phoroslides" which were strips of cellulose acetate membranes bonded to mylar[1]. Four "phoroslides" were marked for identification, prebuffered for 15 minutes in 0.05M barbitone buffer (BB) at pH 8.6 then inserted into a four-cell electrophoretic cell[1] containing 40 ml of barbitone buffer at pH 8.6. Sera were applied to the "phoroslides" using sample applicators[1] which consisted of two welded stainless steel strips moulded into a plastic handle to form a space which delivered a sample volume of 0.25 µl. The power module was constant 100 volts. The "phoroslides" were then removed, stained with 1.8 percent Ponceau-S dye[1] in distilled water for ten minutes at room temperature and then destained in a series of three trays containing five percent acetic acid in deionized distilled water. The "phoroslides" were air-dried, dehydrated in ethanol and then cleared in a solution consisting of glacial acetic acid and ethyl acetate (70;30). The cleared strips were dried in a vented oven at 60°C. Densitometric quantitation was performed on the stained "phoroslides" using a phoroscope[1]. (Plate 2 and 3).

Total Serum Protein Concentrations

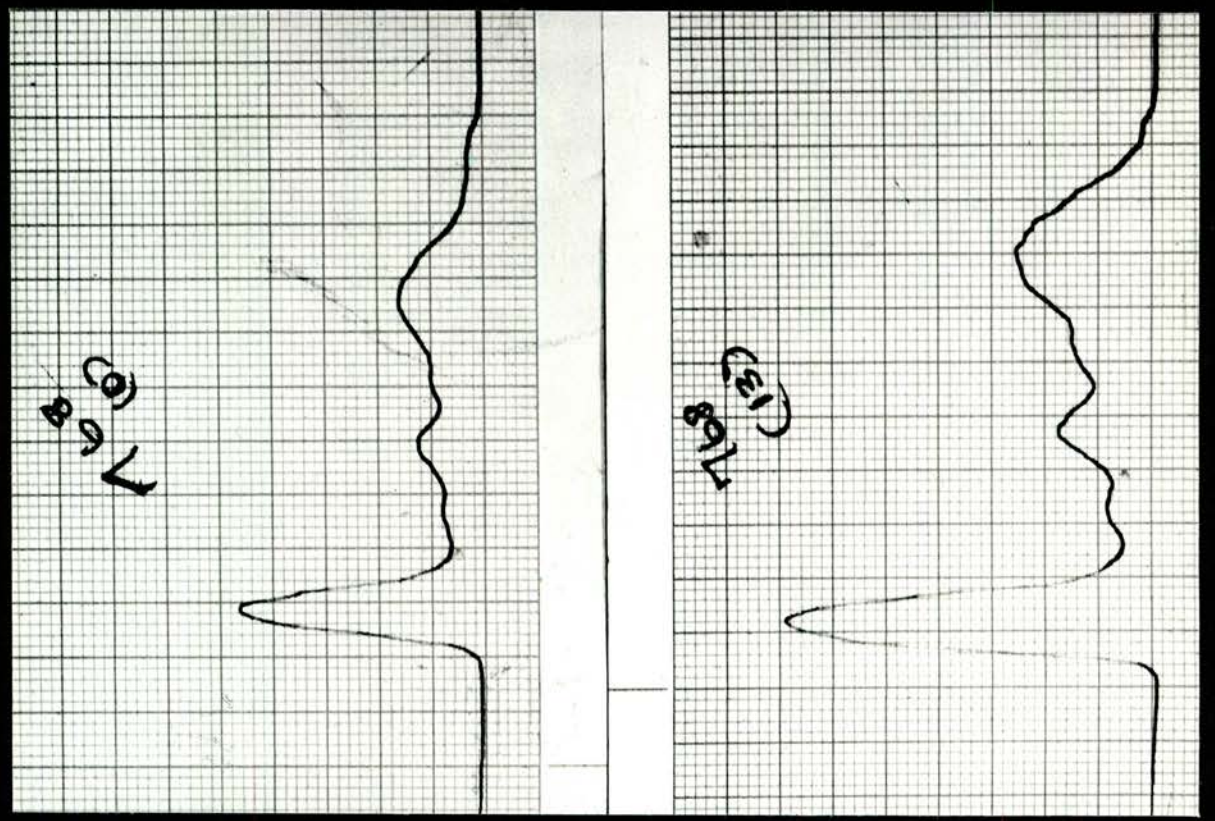
The total serum protein concentrations were determined using the Biuret method. Test tubes were set up

1. Millipore Corporation.

Plate 2 Stained "Phoroslides" -
Sera from sheep No. 768 taken on Day 0,
1, 13 and 14.

Plate 3 Densitometric tracing of the 'phoroslides'
Sera from sheep No. 768 collected on day 0
and 13.


14
15
0
1
768
768


768 (0)
768 (13)

in pairs and the following solutions were added to each:-

4.9 ml of 3 percent sodium hydroxide prepared in distilled water,

0.1 ml of the serum sample or seronorm,[1] a reference protein standard containing 7g/100ml.

1 ml of Biuret reagent, a copper solution made up of 17.3g of copper sulphate, 173g trisodium citrate and 100g sodium carbonate dissolved in one litre of warm deionized distilled water.

The contents of the tubes were thoroughly mixed and the blue concentration allowed to develop for a minimum time of 30 minutes. The absorbances of the standard serum (As) and the serum samples (Ax) were read at 545 nm against the reagent blank in a spectrophotometer[2].

The protein content of the serum samples were then calculated using the equation:-

$$g/100 \text{ ml of serum} = \frac{Ax}{As} \times 7$$

Thereafter the values were translated into g/l.

Immunoglobulin Levels

Levels of various classes of immunoglobulins in the sera from sheep infected with orf virus were determined by single radial immunodiffusion. To carry out single radial immunodiffusion, pure sheep immunoglobulins were isolated and purified then antisera to the immunoglobulins prepared in rabbits and goats as follows:-

1. Nyegaard and Co., Oslo.
2. Unicam, SP 1800.

Isolation and purification of IgM immunoglobulin:

Pure IgM was prepared by the precipitation of euglobulins from pooled normal sheep serum and by the fractionation of the dissolved euglobulins by gel-filtration chromatography. A euglobulin fraction assumed to contain most of the IgM was precipitated by dialyzing 50 ml of serum in Visking tubing[1] against running tap water at room temperature for 72 hours. The precipitate was harvested by centrifugation at 1,600 g for 15 minutes at 4°C. The precipitate was then dissolved in four ml of 0.1M Tris[3]-NAOH buffer at pH 8.0. Sephadex G200[2] was used for the gel-filtration chromatography. Twenty g of Sephadex G200 was left in 750 ml of Tris-NAOH buffer at pH 8.0 for five hours in a boiling water bath. The gel slurry formed was allowed to cool and was then degassed before packing into column to form a gel bed of 90 cm long and 2.5 cm diameter. The column was connected to the LKB unit[3], was checked for homogeneity by running through two ml of 0.2 percent Blue Dextran 2000[2]. Two ml of the dissolved euglobulin sample was applied to the column and fractionation allowed to proceed at a flow rate of 20 ml per hour. The first peak, which contained most of the IgM was collected in test tubes and a further two ml sample was fractionated. The collected eluent was concentrated by

1. Visking tubing - Medicell International Ltd.
2. Pharmacia. 3. LKB unit consisted of peristatic pump (Varioperpex 12000) control unit (Uvicord II 8300) fraction collector (Ultrac 7000 and chromatogram recorder type 6520.

dialysis against 40 percent polyethylene glycol 6000[1].

Isolation and purification of IgG1, and IgG2: Pure IgG1 and IgG2 were prepared by the precipitation of globulin fractions of pooled normal sheep serum and then by fractionation of the dissolved precipitate in ion-exchange chromatography. The globulin fraction assumed to contain most of the serum immunoglobulins was precipitated by adding, dropwise, an equal volume of 41 percent saturated ammonium sulphate at pH 6.5. The mixture was stirred continuously at room temperature for 30 minutes then was allowed to stand at 4°C overnight. The precipitate was harvested after centrifugation at 2,800 g[2] for 20 minutes, washed once in saturated ammonium sulphate and then dissolved in 0.01M phosphate buffer (PB) at pH 7.4. The fraction provided the starting material for preparing pure IgG1 and IgG2 by ion-exchange chromatography. Diethylaminoethyl cellulose[3] was equilibrated in 0.01M PB then packed into a column of 40 cm long and 2.5 cm diameter which was connected to the LKB unit. The re-dissolved ammonium sulphate-precipitated globulin fraction was dialyzed against PB for 24 hours at 4°C and then loaded onto the column. Separations of the IgG1 and IgG2 were carried out by stepwise elution. IgG2 was eluted first with 0.01M PB and IgG1 using 0.05M PB. The eluted samples were concentrated by dialysis against 40 percent polyethylene glycol. The IgG1 and

1. Carbowax - BDH.
2. 4,000 rpm in Minifuge Hereaves Christ.
3. DE 52, Whatham.

IgG2 elutes were purified further by re-fractionation once on DE 52 and once on Sephadex G200 column.

Immunoelectrophoresis: The purity of the isolated immunoglobulin fractions was checked by immunoelectrophoresis. Square glass plates (6 x 6 cm) were filled with melted 1.5 percent Special agar[1] in barbital buffer at pH 8.6 and allowed to solidify at room temperature. A well-trough pattern was punched out with the help of a template and the agar plugs were removed from the wells into which the different isolates were added. The plates were then placed in a tray of an electrophoretic chamber[2] filled with barbital buffer at pH 8.6. Moist wicks made out of filter paper were attached to the edges of the gel into the buffer thus creating electrical continuity. A current of 100 volts was applied for 60 minutes. The plates were then removed and placed in a moist chamber. Rabbit anti-whole sheep serum was placed in the troughs and diffusion was allowed to proceed at room temperature overnight. The plates were then washed in saline and the precipitation lines observed (Plates 4-6).

Protein content of the purified immunoglobulins: Determination of the protein content of the purified sheep IgM, IgG1 and IgG2 was carried out using Folin and Ciocalteau's reagent[3]. Volumes of 0.2ml of a protein standard[4] or the test samples were mixed thoroughly with 10 ml of alkaline copper solution (5ml of 0.1 percent $CuSO_4$ mixed with 45 ml of alkaline tatrate) in a test-

1. Oxoid Ltd.
2. LKB.
3. BDH Chemicals.
4. Difco laboratories, Detroit, U.S.A.

Plate 4 IgM - A - well with whole sheep serum.

B - well with isolated sheep IgM.

Trough - Rabbit anti-whole sheep serum.

Plate 5 IgG_1 A - well with whole sheep serum.

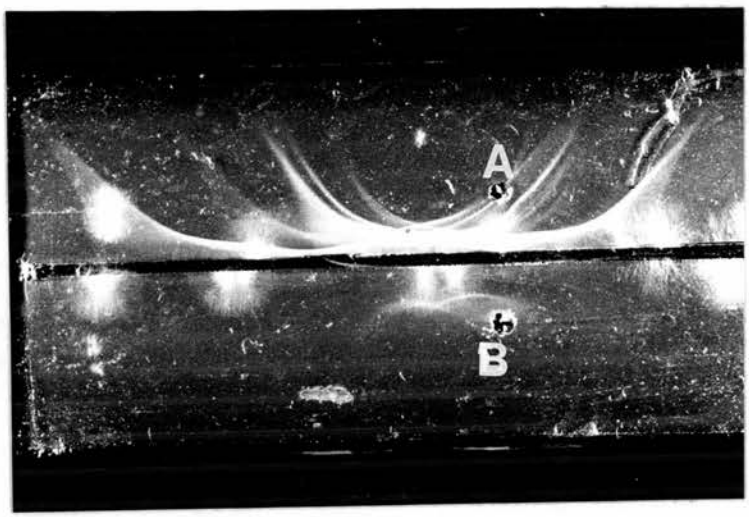
B - well with isolated sheep IgG_1.

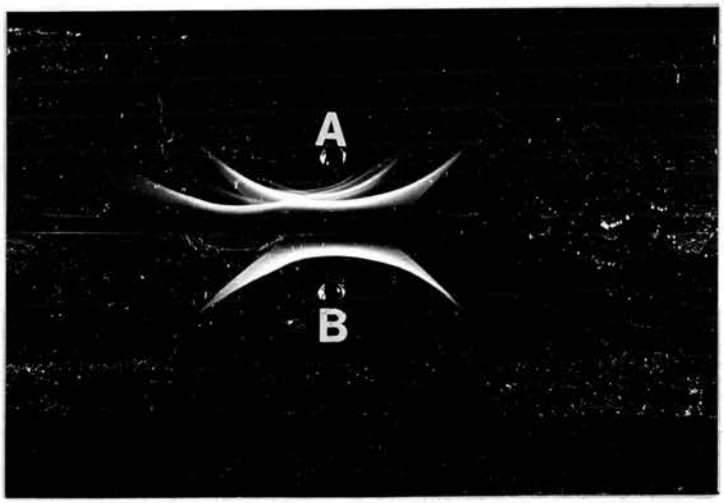
Trough - Rabbit anti-whole sheep serum.

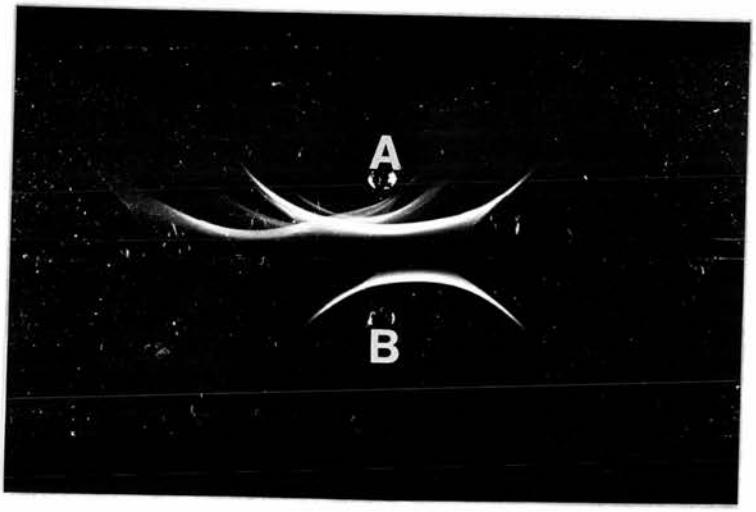
Plate 6 IgG_2 A - well with whole sheep serum.

B - well with isolated sheep IgG_2.

Trough - Rabbit anti-whole sheep serum.


A
B


A
B


A
B

tube and allowed to react for 15 minutes. Concentrated Folin's and Ciocalteau's reagent was diluted 1/3 in DDW and one ml of the diluted reagent was added to the test-tubes. The tubes were left at room temperature for 30 minutes to allow the dark blue colouration to develop. The absorbance of the blue colour was read at 750 nm in a spectrophotometer against a reagent blank. The protein content was calculated using the following equation:-

$$g/100\ ml = \frac{\text{Absorbance in the test samples}}{\text{Absorbance in the standard protein}} \times 7$$

Thereafter the results were translated into g/l:-

Immunoglobulin Fractions		
Igm	IgG_1	IgG_2
1.44 g/l	2.2	3.7

Antisera preparation: Antisera to sheep IgM and IgG1 were raised in mature New Zealand white rabbits by Mr. Ali Shubber. He injected each rabbit intramuscularly with 2 mg of purified sheep IgM or IgG1 thoroughly mixed with Freund's complete adjuvant[1]. He repeated the injections four times at two weeks intervals. I administered booster doses to these rabbits and bled them one week later. I harvested the sera and checked for antibody titres using the Ouchterlony gel-diffusion test. If the titre of the antiserum was at least 1/32 more blood was collected; if not the rabbits were given

1 Difco Laboratories, Detroit U.S.A.

further booster doses of appropriate antigens in complete Freund's adjuvant. Enough rabbit anti-sheep IgA serum was kindly donated by Mr. Ali Shubber and this was used in the preliminary test.

Similarly, the IgG2 antiserum was raised in two goats which were first immunized by Mr. Ali Shubber who had injected each with 5 mg/kg body weight of pure sheep IgG2 mixed thoroughly in complete Freund's adjuvant. I gave these goats booster doses twice at a two week interval and bled them one week later to check for antibody titre. If the titre was 1/32 then more blood was taken for antiserum. The different antisera were made monospecific by a series of cross-absorptions with appropriate antigen in order to remove anti-light chain activity. Antiserum to IgM was absorbed using 2 mg/ml of pure IgG1 and 2 mg/ml of IgG2, antiserum to sheep IgG1 was absorbed using 2 mg/ml of pure sheep IgG2; antiserum IgG2 was absorbed by 2 mg/ml of IgG1 (Plates 7 and 8).

Ouchterlony double diffusion test: The titres of the antibody in the antisera prepared in the rabbits and goats was determined by double diffusion in agar gel. Seven ml of melted 2 percent Special agar was pipetted onto petri dishes or slides precoated with 0.3 percent agar on a levelled surface then allowed to solidify. Seven wells were punched out using a standard template and the gel plugs removed from the wells by suction. The wells were filled carefully with sheep serum in the central well and

Plate 7 Antisera Production to IgM and IgG_2

All Wells had whole sheep serum

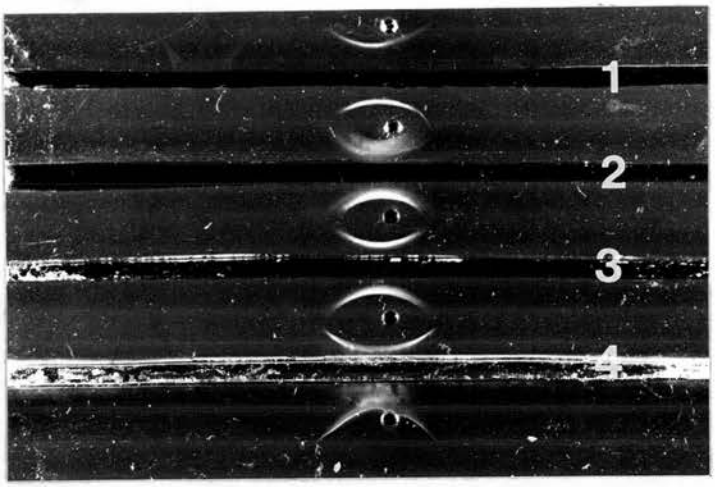
Troughs 1 and 3 - antisera to sheep IgG_2

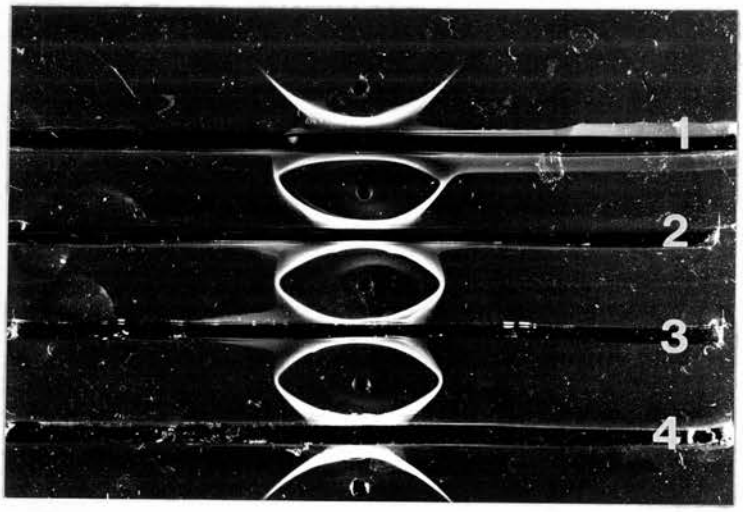
Troughs 2 and 4 - antisera to sheep IgM.

Plate 8 Antisera Production to IgG_1

All wells had whole sheep serum

Troughs 1 - 4 had antisera to sheep IgG_1.


1
2
3
4


1
2
3
4

different dilutions of the antiserum in the outside five wells. The sixth well was filled with 0.15 percent of sodium chloride. The slides or petri dishes were then placed in moist chamber and incubated at room temperature for 24 hours. The precipitation lines were read with the help of an oblique light viewer[1]. The double diffusion test was also used in the preliminary tests carried out to determine the changes in the levels of various immunoglobulins in the sera from the sheep experimentally infected with orf virus.

Single radial immunodiffusion: The levels of various immunoglobulins in the sera were determined by a single radial immunodiffusion method first described by Mancini, Carbonara and Heremans (1965) and later modified by Fahey and McKelvey (1965). Melted 3 percent agarose[2] in phosphate buffered saline (PBS) at pH 7.3 was mixed with an equal volume of the appropriate rabbit or goat antiserum warmed to 56°C and then poured onto glass plates (10 x 10 cm) on a spirit-levelled stand. The antisera to IgG1 and IgG2 were diluted 1/10 and the antiserum to IgM and IgA were diluted 1/5 in PBS before heating to 56°C and mixing with the melted agarose. The agarose-antiserum mixture on the plates was allowed to solidify then, using a template, 81 wells were cut in the gel and the agar plugs removed from the wells by suction. Each well was filled with 2 µl of the test ovine serum diluted 1/4 for the determination of the levels of IgM and IgG2

1. Luckham Ltd.
2. BDH Chemicals.

1/32 for the determination of the levels of IgG1. A standard reference serum diluted 1/2, 1/4, 1/8 and 1/32 was put in the first four wells in the top left-hand corner. The plates were then incubated in humid chamber at room temperature for 24 hours for IgG1, IgG2 and IgA and for 48 hours for IgM. The diameter of the precipitin rings were measured using a calibrated magnified viewer[1] / (Plates 9 - 11). The results were plotted on a linear graph as a function of the reference serum concentration. The concentrations of the test ovine sera were then calculated from a linear regression derived by the method of least squares (Snedecor and Cochran, 1974).

The standard reference serum was made up of 0.1 ml of serum from all the animals whose serum samples were to be quantitated. This pooled serum was first tested against the known concentrations of the purified IgM, IgG1 and IgG2 and the concentrations of various immunoglobulin classes were determined as described above and were as follows:-

IgM	IgG_1	IgG_2
5 g/l	20	6.5

Passive Haemagglutination Tests

The passive haemagglutination tests were carried out to detect orf antibodies in the sera from the sheep experimentally infected with orf virus.

1. Nikon

Plate 9 Single Radial Immunodiffusion

IgM determination.

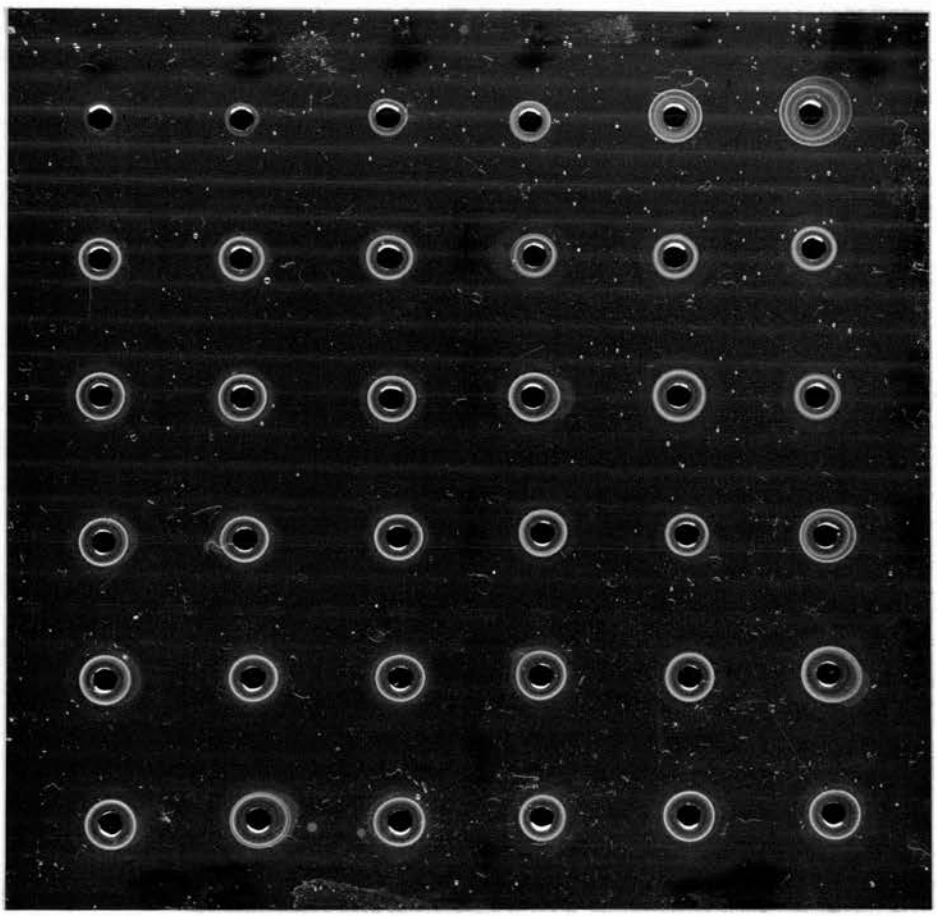


Plate 10 Single Radial Immunodiffusion

IgG_1 determination.

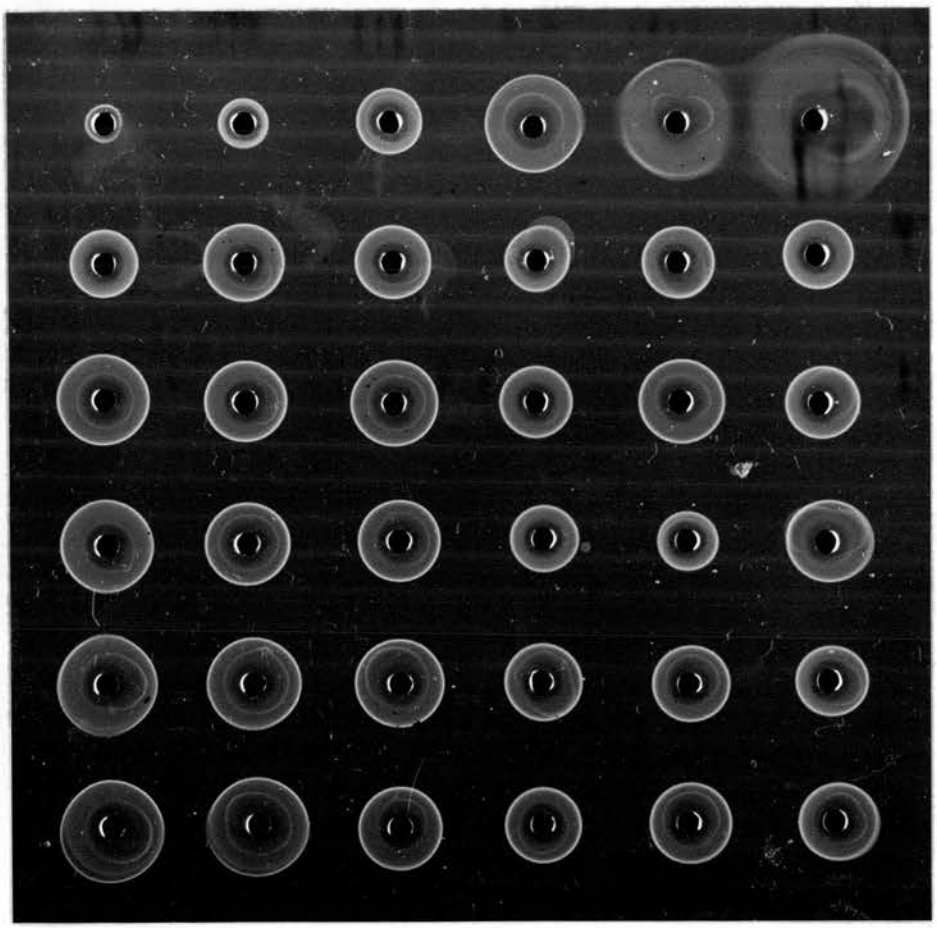
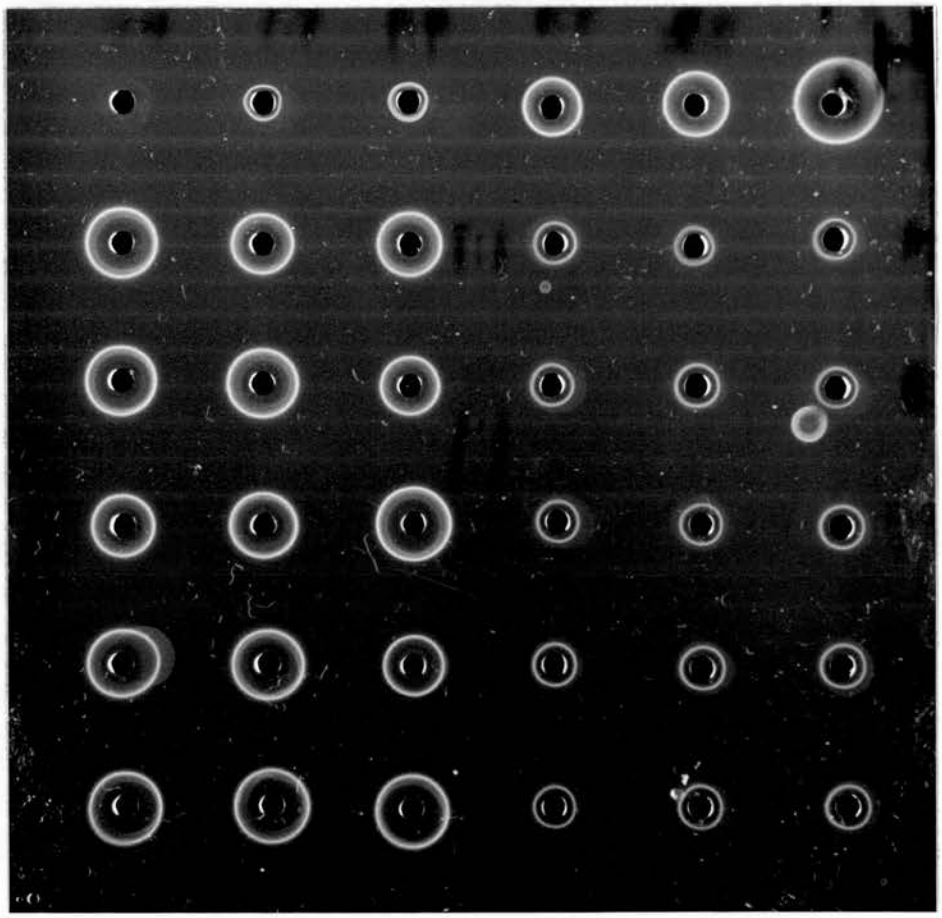


Plate 11 Single Radial Immunodiffusion

IgG_2 determination.



Preparations of orf antigen: Orf virus passaged eight times in cultures of lamb testis cells and five times in the cultures of calf testis cells was concentrated from 50 ml to 25 ml by dialysis against 40 percent polyethylene glycol. The virus particles in the concentrated fluid were sedimented by ultracentrifugation at 78,624 g[1] for 30 minutes. The supernatant fluid containing the soluble antigens was dialyzed against phosphate buffer at pH 7.4 for 24 hours at 4°C. This solution was clarified by centrifugation at 1,600g for 30 minutes. The protein content of this antigen preparation determined using Folins' phenol reagent was 30.4 mg/100 ml. The antigen preparation was then stored in a plastic universal bottle at - 20°C until required.

Sensitization of the erythrocytes: Sheep erythrocytes were collected in a 50 ml "Vacutainer" containing 20 ml of Alsever's solution[2] and stored at 4°C for three days. The cells were washed three times in 0.15 percent sodium chloride and three times in PB at pH 7.4 before they were diluted in PB to a final concentration of 2.5 percent. Twentyfive ml of the erythrocyte suspension was then mixed with 3 ml of 2.5 percent glutaraldehyde in PB and 15 mg of the soluble antigen solution was added immediately. Sensitization was allowed to proceed at 37°C with gentle magnetic stirring for 60 minutes by which time the cells had turned chocolate brown from their normal bright red colour. The coated cells were then washed three times in

1. Beckmann, Model L2-65B.
2. See Appendix II for composition.

PB and suspended in PB containing 0.2 percent bovine serum albumin[1] as a stabilizer and 0.02 percent sodium azide as an antibacterial agent. Control cells were similarly treated with glutaraldehyde but antigen was not included. The final suspension of the antigen coated cells and the control cells were stored at 4°C for three months.

The tests: The test was carried out in V-shaped 96-well microtitre plates[2]. The test serum samples were inactivated by heating in waterbath at 56°C for 30 minutes after which they were absorbed by adding 0.3 ml of 20 percent concentration of control cells to 0.1 ml of serum. A series of two-fold dilutions of each absorbed serum sample was then made in PB in the microtitre plates and an equal volume of the coated cells at a concentration of 0.6 percent was added to each well. A sample of serum from a sheep known not to possess antibodies to orf virus, a sample of positive serum and erythrocytes not coated with antigen but treated with glutaraldehyde were included as controls. Antibody titrations were conducted using 2-fold dilutions steps. The titration end-point was taken to be the dilution giving 50 percent agglutination and it was expressed as the negative logarithm to the base two.

1. Sigma Ltd.
2. Sterilin.

RESULTS

Serum Protein Patterns of Sheep Challenged with Orf Virus

Group I: Estimations of the total protein contents of the sera from 16 sheep previously infected with orf virus ranged from 54 to 86 g/l and in the samples taken after challenge ranged from 52 to 116 g/l (Appendix Table I). The mean values of daily samples ranged from 73 $\pm$ 14 to 83 $\pm$ 8.3 g/l. The mean differences between the pre- and post-challenge values were not significant (Table I).

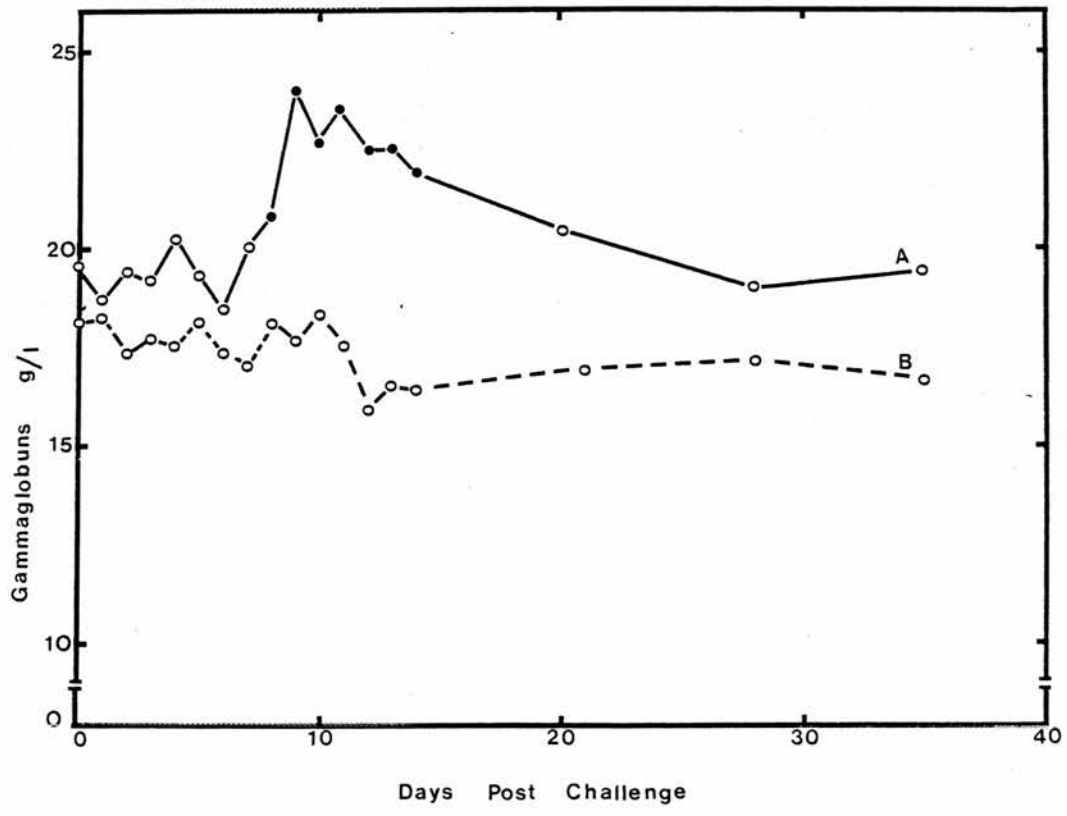
Electrophoresis of the same serum samples resolved the serum protein components into five readily discernible bands conventionally attributed to albumin and alpha-1, alpha-2, beta- and gamma-globulins. The calculated serum protein values are shown in Appendix Tables 2 - 6. The mean differences between pre-and post-challenge values of albumin and alpha-1 alpha-2 and beta-globulins were not significant (Tables 2 - 5). In contrast, the mean differences between the pre- and post-challenge values of the gamma-globulin fractions were significantly greater on days 9 to 14 post-challenge (Table 6). A plot of the daily means of gamma-globulin values reflects this trend (Figure I): the mean gamma-globulin levels in the pre-challenge sera was 19.5 $\pm$ 8.0 g/l. The levels increased gradually after challenge to reach a peak value of 24.0 $\pm$ 9.2 g/l on the ninth day, then decreased to the

Figure 1

The daily means of gammaglobulin levels of:-

A - Previously infected sheep challenged with orf virus (Group 1).

B - Previously infected sheep not challenged (Group 2).



pre-challenge value by day 35, the day the experiment was terminated.

Group 2: The estimates of the total protein contents of the sera from five previously infected sheep which acted as an uninfected control group ranged from 61 to 76 g/l (Appendix Table 11). The mean values of the daily samples ranged from 63.6 $\pm$ 1.34 to 69.2 $\pm$ 3.77 g/l. The mean value of the samples taken at the onset of observations was not different from the mean value of the pre-challenge samples of the previously infected sheep in the group I ($t_{(19)} = 1.77$; $P > 0.2$). There were significant changes in the mean differences between the group 2 samples taken at the onset of observations and the samples taken on 2, 3, 4, 5, 12, 14 and 21 days later (Table 7).

The densitometric evaluations of the serum protein fractions in the same samples are given in Appendix Tables 12-16. The mean differences between the values at the on-set (albumin and alphal-, alpha2-, beta- and gamma-globulin) of observations and during the subsequent 35 days were not significant (Tables 8-12). Comparison of the daily gammaglobulin values between the challenged and unchallenged previously infected sheep is shown in Table 13 and Figure I.

Group 3: Estimations of the total protein contents of the sera from eight susceptible sheep challenged with orf virus ranged from 67 to 81 g/l in the sera taken before challenge and 63 to 81 g/l in the sera taken after challenge. The mean values of daily samples ranged from 66.2 $\pm$ 4.7 to 72 $\pm$ 3.0 g/l (Appendix Table 21). The mean value of the pre-challenge samples was similar to

the mean value of the previously infected sheep and mean values of previously infected sheep which were not challenged ($t_{(22)} = 0.945$, P 0.30 and $t_{(11)} = 0.784$, P 0.40). respectively. The mean differences between pre- and post-challenge values were not significant (Table 14).

Electrophoresis evaluations of the serum proteins in the same serum samples are shown in Appendix Tables 22-26. The mean differences between the pre- and post-challenge values of albumin and alphal-, alpha2-, beta- and gammaglobulins were not significant (Tables 15-19). Comparison of the daily mean values of gamma-globulins of the challenged susceptible and previously infected sheep is shown in Table 20 and Figure 2.

Immunoglobulin Concentrations

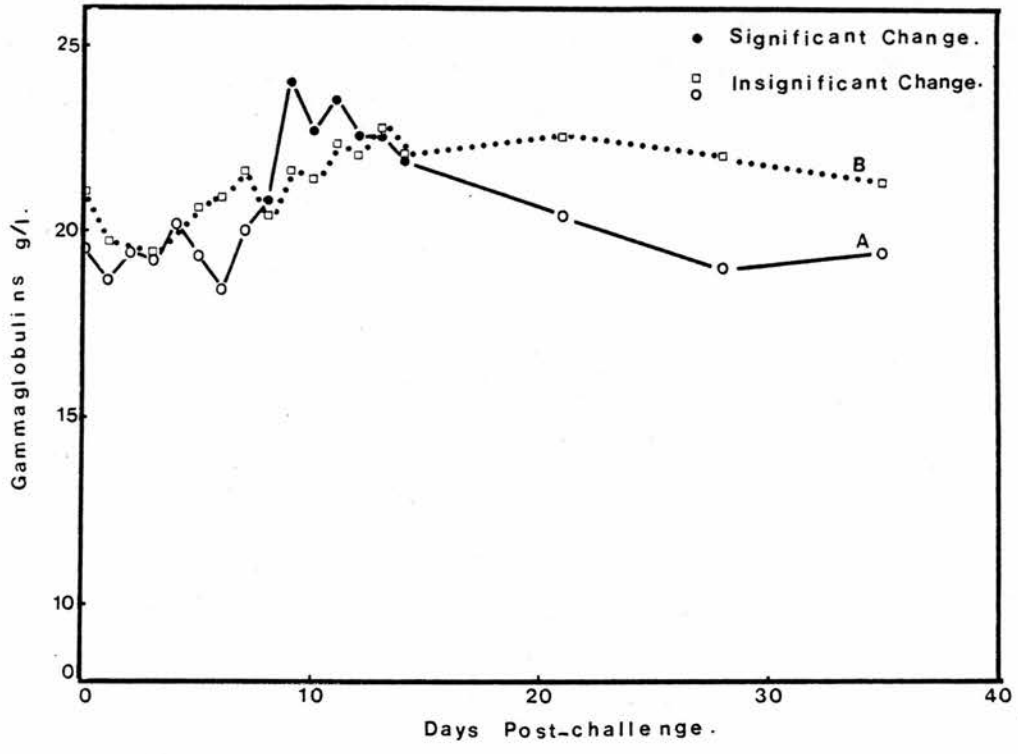
A preliminary test was carried out to check the changes in the different immunoglobulin classes and subclasses in the sera from sheep of the previously infected group which was challenged with orf virus. There was a clear indication of changes in the IgM, IgG1 and IgG2 levels between the serum samples collected before challenge and during the course of the infection, but there were indetectable changes in the levels of IgA therefore, changes in this class of immunoglobulin were not studied in the subsequent experiments.

Group I: Changes observed in IgM, IgG1 and IgG2 levels in the serum samples collected from the 16 previously infected sheep which were challenged with orf virus are shown in Figure 3 and Appendix Tables 7 - 9.

Figure 2: The daily means of gammaglobulin levels in sheep infected and re-infected with orf virus.

A: Re-infected sheep (Group I).

B: Infected susceptible sheep (Group 3).



The mean level of IgM in the pre-challenge sera was 3.6 ± 1.0 g/l and after challenge the mean level rose to 4.5 ± 1.6 g/l on the second, third and fourth day but decreased to pre-challenge levels by the seventh day after challenge (Table 21 and Figure 3).

The IgG1 levels in the same serum samples rose from pre-challenge mean value of 15.8 ± 6.9 g/l to a peak value of 22.2 ± 9.2 g/l on the 12th day after challenge, thereafter decreasing gradually. The changes in the IgG1 levels were statistically significant from days 11 to 35 after challenge (Table 22 and Figure 3).

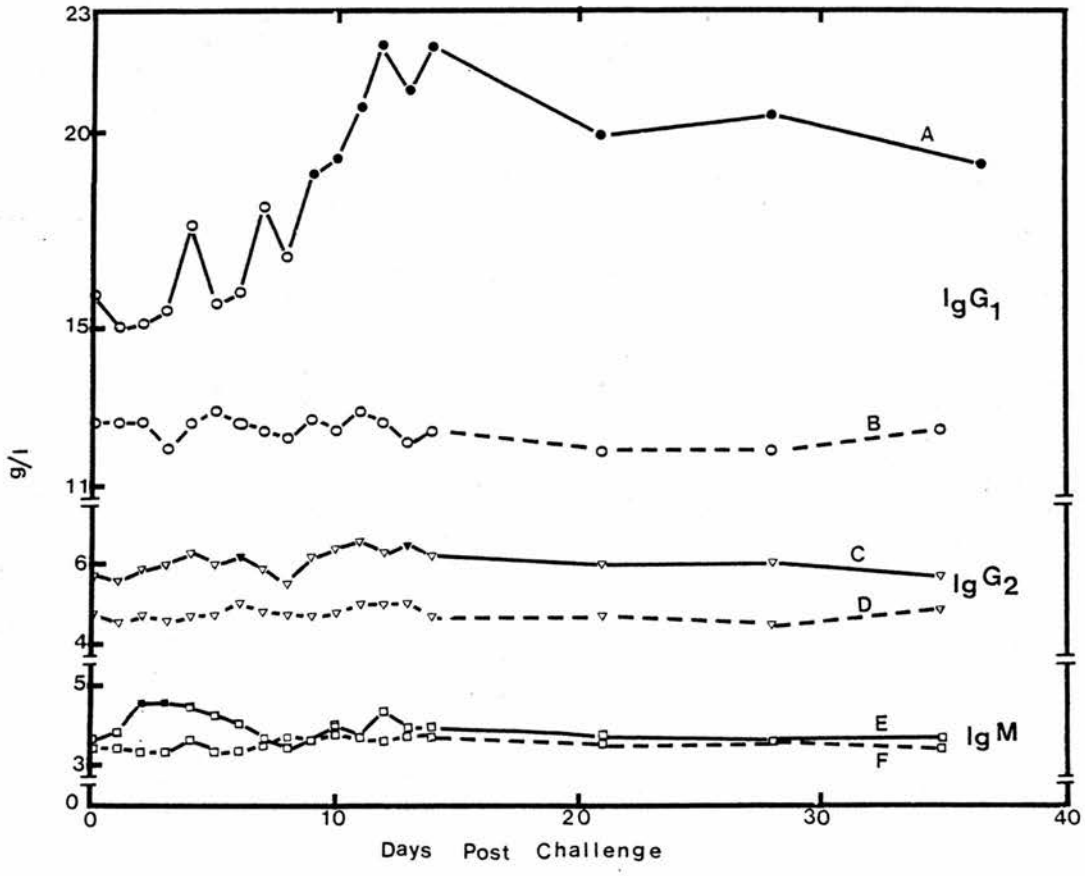
Similarly, IgG2 levels in the same serum samples rose from pre-challenge mean value 5.7 ± 2.7 g/l to 6.6 ± 3.1 g/l on day 11 after challenge and these levels decreased gradually to the levels before challenge by day 35 (Table 23 and Figure 3).

Group 2: No significant changes were found in the levels of IgM, IgG1 and IgG2 in the sera from the five previously infected sheep that were not challenged (Tables 24-26 and Figure 3). The IgM levels ranged from 3.28 ± 0.63 to 3.76 ± 0.88 g/l; IgG1 levels ranged from 11.92 ± 1.24 to 12.86 ± 1.02 g/l and for IgG2 the levels ranged from 4.52 ± 0.4 to 4.99 ± 0.55 g/l (Appendix Tables 17-19).

Comparison between the daily means of the IgG1 of the challenged and un-challenged previously infected sheep revealed significant increases in the values in the challenged sheep particularly after the tenth day of challenge

Figure 3 The daily means of the IgM, IgG_1 and IgG_2 levels in challenged and unchallenged previously infected sheep.

A - IgG_1 levels in challenged sheep (Group I)
B - IgG_2 levels in unchallenged sheep (Group 2)
C - IgG_2 levels in challenged sheep (Group 1)
D - IgG_2 levels in unchallenged sheep (Group 2)
E - IgM levels in challenged sheep (Group 1)
F - IgM levels in unchallenged sheep (Group 2).



(Table 27 and Figure 3). Comparisons of the IgM and IgG2 levels between the two groups were not significant (Tables 28 and 29).

Group 3: Only slight changes which were not significant were noted in the levels of IgM. The IgM mean levels ranged from 3.05 g/l at day 0 to 3.3 g/l on the sixth day after challenge and the IgG2 mean levels ranged from 4.4 $\pm$ 2 g/l in the pre-challenge sera to 5.2 $\pm$ 2.2 g/l, highest value recorded on day 14 post-challenge. The increases in IgG2 levels were significant on days 14, 21 and 35. (Tables 30 and 32, Figure 4 and Appendix Tables 28 and 29).

The IgG1 levels in the same serum samples ranged from 11.5 $\pm$ 5.9 g/l in the sera collected before challenge to 13.6 $\pm$ 5.6 g/l on day ten after challenge. The increase in the IgG1 levels was statistically significant on day 11 (Table 31 and Figure 4 Appendix Table 29).

Comparison between IgG1 values of unchallenged susceptible and previously infected sheep revealed that there was a significant difference from day 12 through to day 35 (Table 33). In contrast, the comparison of IgM and IgG2 levels were not significant (Tables 34-35).

Orf Antibodies

Orf antibody titres varied from group to group, animal to animal, and from day to day, but, in general, higher titres occurred in previously infected sheep challenged with orf virus than in susceptible sheep with a primary orf infection (Table 36).

Figure 4: The daily means of the IgM, IgG_1 and IgG_2 levels in the sheep infected and re-infected with orf virus.

A - IgG_1 levels in re-infected sheep (Group 1).

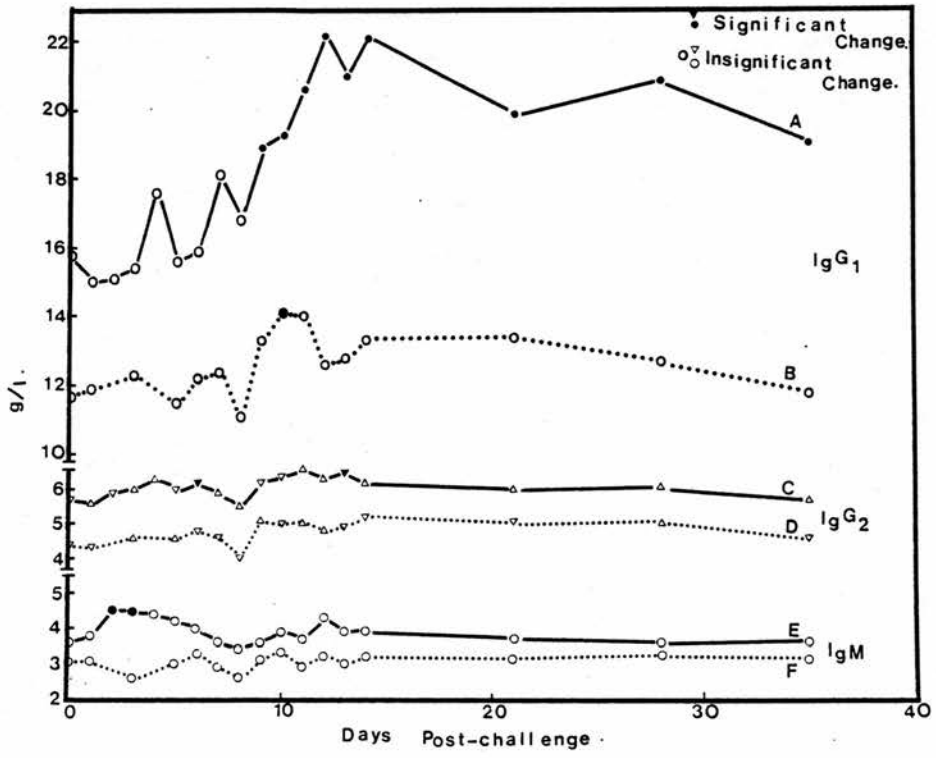
B - IgG_1 levels in infected susceptible sheep (Group 3).

C - IgG_2 levels in re-infected sheep (Group 1).

D - IgG_2 levels in infected susceptible sheep (Group 3).

E - IgM levels in re-infected sheep (Group I).

F - IgM levels in infected susceptible sheep (Group 3).


Significant Change.
Insignificant Change.
A
B
C
D
E
F
IgG1
IgG2
IgM
g/l.
Days Post-challenge.
22
20
18
16
14
12
10
6
5
4
5
4
3
2
0
10
20
30
40

Group 1: The rise in orf antibody titres detected in the 16 previously infected sheep challenged with orf virus is illustrated in Figure 5. Eight of the previously infected sheep had detectable orf antibodies in the pre-challenge sera indicating previous infection. After challenge, the antibody titres rose progressively in all re-infected sheep reaching the highest mean value of 4.62 ± 1.1 on day 13. Thereafter, the titres gradually decreased to 3.62 ± 0.88 on day 35, the day the experiment was terminated. The mean increase in the antibody titres was statistically significant on days 8 to 35 (Table 37).

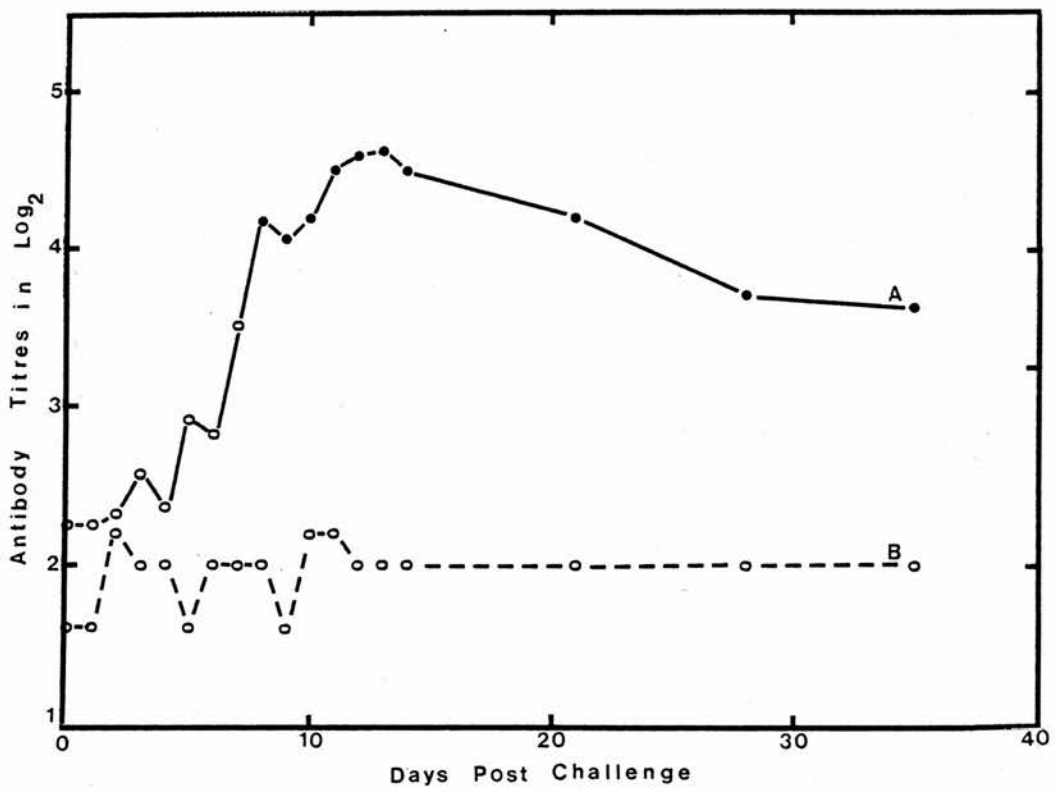
Comparing the dynamics of the antibody responses of previously infected sheep which had detectable antibodies in the pre-challenge sera, with those of the sheep which had no antibodies or only traces of antibodies revealed that the slopes were similar but the positions of the lines were significantly different (~~Figure~~ $F_{12}^{1} = 2.36$; $P > 0.05$). Similarly, there was no significant difference in the slopes of the exponential declines (~~Figure~~ $F_{6}^{1} = 0.43$; $P > 0.05$ Figure 6 Appendix Tables 10, 10a, and 10b).

The correlation between mean daily gammaglobulin levels and mean daily antibody titres was positive and significant ($r_{(16)} = 0.76$; $P < 0.01$). There was not, however, a significant correlation between the gammaglobulin values and antibody titres of the sheep on specific days. Nevertheless, the correlation between gammaglobulin values and antibody titres in most individual

Figure 5 The daily means of orf antibody titres of:-

A - Previously infected sheep challenged with orf virus (Group 1).

B - Previously infected sheep not challenged (Group 2).



sheep was significant (Table 39).

Likewise, the correlation between the mean IgG1 levels and mean daily antibody titres was positive and significant ($r_{(16)}$ = + 0.864; $P < 0.01$). Similarly there was no significant correlation between the IgG1 levels and antibody titres on specific days although a significant correlation was noted between the IgG1 levels and antibody titres in most individual sheep (Table 41).

Group 2: The orf antibody titres of five previously infected sheep which were not challenged with orf virus ranged from 1.6 to 2 over the period of the experiment (Figure 5). Consequently, when the antibody titres of the daily means of the challenged and non-challenged previously infected sheep were compared, significant differences were detected on days 5, 7 and from day onwards ~~and 8, and from the 12th day onwards~~ (Table 42).

Group 3: None of the pre-infection serum samples from eight susceptible sheep had measurable orf antibodies. Changes were minimal during the first week of infection but, thereafter, the antibody titres rose to 2.7 on day 12 and remained more or less at the same level up to day 35.

There was a positive and significant correlation between the daily mean levels of gammaglobulin and the daily mean antibody titres ($r_{(14)}$ = + 0.67; $P < 0.01$) and between the daily mean levels of the IgG1 and the daily mean antibody titres ($r_{(14)}$ = + 0.67; $P < 0.01$). There was no significant correlation between the gammaglobulin values and antibody titres on specific days

(Tables 43 and 44). Nevertheless, the correlation between gammaglobulin and antibody titres and between IgG1 values and antibody titres in most sheep were positive and significant (Tables 43 and 44).

The rate of antibody production in the infected susceptible sheep was significantly slower than in the challenged previously infected sheep (Figure 6) ($F^{2}_{21} = 7.56$; $P < 0.01$). The "onset" of orf antibody production in infected susceptible sheep was 7.6 ± 3.6 days after infection and in the challenged previously infected sheep was 5.3 ± 2.6 days after challenge; the difference was not statistically significant ($t_{(21)} = 1.697$; $P > 0.20$). The peak titres of orf antibody were detected earlier in the challenged previously infected sheep than in the infected susceptible sheep, namely, on days 10.3 ± 2 and 23.1 ± 11 respectively. The difference was highly significant ($t_{(13)} = 3.30$; $P < 0.01$).

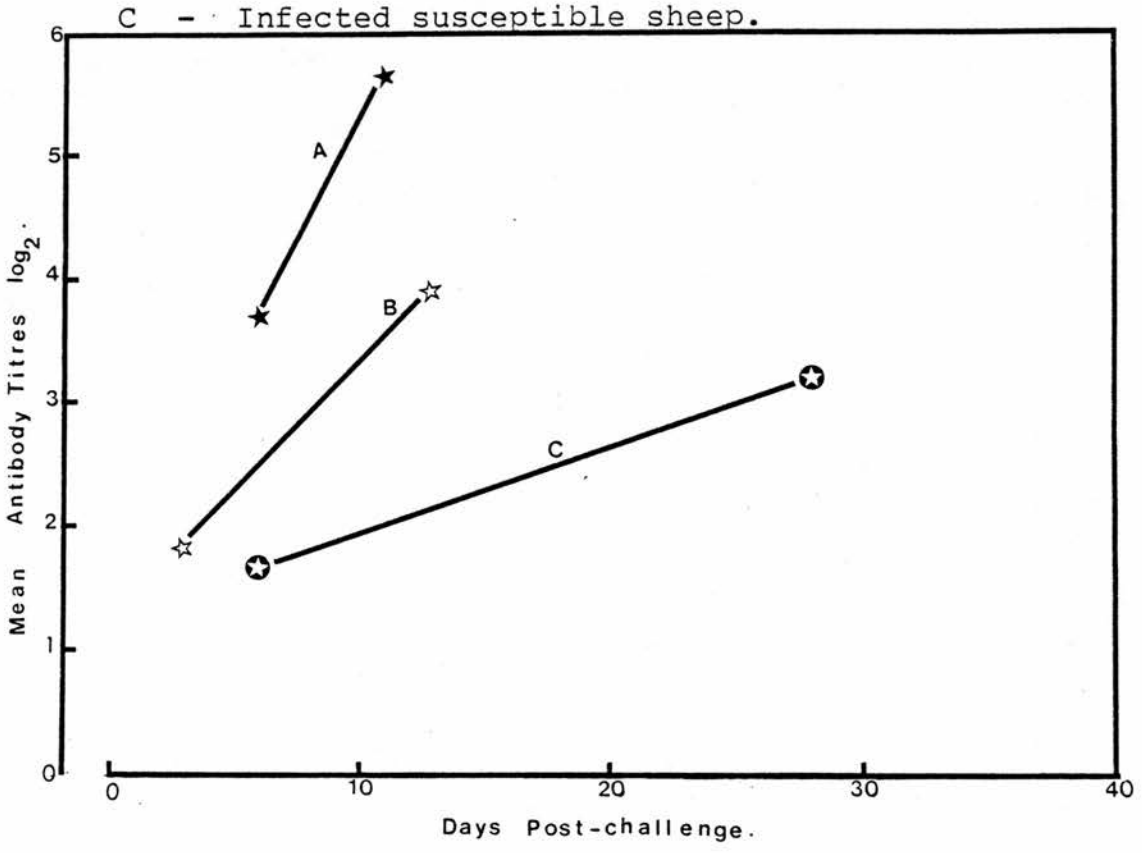
The antibody titres of the previously infected sheep were significantly higher than the titres of the susceptible sheep after challenge (Table 36 and Figure 7).

Figure 6: The rate of orf antibody production in sheep infected and re-infected with orf virus.

A - Re-infected sheep with detectable antibodies before challenge.

B - Re-infected sheep without detectable antibodies.

C - Infected susceptible sheep.



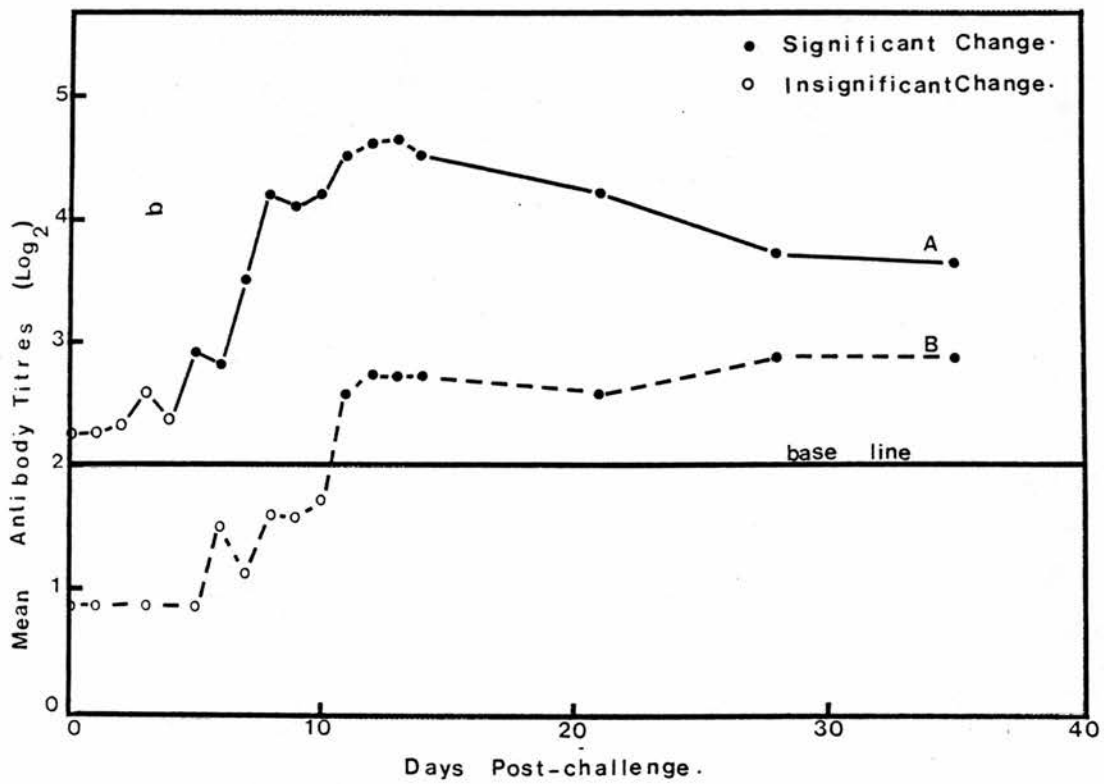


Figure 7: The daily means of orf antibody titres of:-

A - Previously infected sheep challenged with orf virus (Group I).

B - Susceptible sheep infected with orf virus (Group 3).

TABLE I.

MEAN DIFFERENCE OF TOTAL SERUM PROTEIN CONTENTS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation.
1	+ 0.88	2.23	0.39	>0.80	N.S.
2	- 7.17	3.71	1.93	>0.10	N.S.
3	- 0.83	3.08	0.27	>0.80	N.S.
4	+ 0.38	1.86	0.20	>0.90	N.S.
5	- 4.00	2.84	1.41	>0.20	N.S.
6	- 1.50	1.76	0.85	>0.50	N.S.
7	- 0.50	3.07	0.16	>0.90	N.S.
8	- 2.50	2.31	1.08	>0.30	N.S.
9	- 6.56	3.20	2.05	>0.10	N.S.
10	- 2.50	2.76	0.91	>0.40	N.S.
11	- 5.31	3.25	1.63	>0.20	N.S.
12	- 8.25	3.38	2.44	<0.05	S.
13	- 5.06	2.59	1.95	>0.10	N.S.
14	- 3.69	2.04	1.81	>0.10	N.S.
21	- 1.56	2.57	0.61	>0.60	N.S.
28	- 0.81	3.30	0.25	>0.90	N.S.
35	+ 0.63	2.60	0.24	>0.90	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 2.

MEAN DIFFERENCE OF ALBUMIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.04	1.40	0.03	> 0.90	N.S.
2	- 1.03	1.97	0.52	> 0.70	N.S.
3	+ 1.53	1.35	1.13	> 0.30	N.S.
4	- 3.08	1.47	2.10	> 0.10	N.S.
5	- 0.07	1.70	0.04	> 0.90	N.S.
6	- 0.79	1.43	0.55	> 0.60	N.S.
7	+ 1.62	2.04	0.79	> 0.50	N.S.
8	- 1.22	1.47	0.83	> 0.50	N.S.
9	- 1.63	1.54	1.05	> 0.40	N.S.
10	0.00	1.85	0.00	> 0.90	N.S.
11	- 0.49	1.92	0.26	> 0.80	N.S.
12	- 2.56	2.15	1.19	> 0.30	N.S.
13	- 2.58	1.55	1.66	> 0.20	N.S.
14	- 1.03	0.94	1.10	> 0.40	N.S.
21	+ 0.09	1.85	0.05	> 0.90	N.S.
28	- 1.72	1.65	1.04	> 0.40	N.S.
35	+ 0.15	1.36	0.11	> 0.90	N.S.

N.S. = Not Significant.

TABLE 3.

MEAN DIFFERENCE OF ALPHA$_1$-GLOBULIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.29	0.37	0.79	>0.50	N.S.
2	+ 0.18	0.64	0.27	>0.80	N.S.
3	+ 0.25	0.57	0.45	>0.70	N.S.
4	+ 0.54	0.46	1.17	>0.30	N.S.
5	+ 0.10	0.54	0.18	>0.90	N.S.
6	+ 0.81	0.51	1.58	>0.20	N.S.
7	+ 0.54	0.58	0.93	>0.40	N.S.
8	+ 0.79	0.53	1.49	>0.20	N.S.
9	- 0.53	0.44	1.22	>0.30	N.S.
10	+ 0.23	0.46	0.50	>0.70	N.S.
11	+ 0.16	0.53	0.30	>0.80	N.S.
12	- 0.28	0.61	0.46	>0.70	N.S.
13	- 0.04	0.44	0.08	>0.90	N.S.
14	- 0.31	0.52	0.59	>0.60	N.S.
21	+ 0.31	0.43	0.71	>0.50	N.S.
28	+ 0.14	0.52	0.28	>0.80	N.S.
35	- 0.08	0.48	0.17	>0.90	N.S.

N.S. = Not Significant.

TABLE 4.

MEAN DIFFERENCE OF ALPHA$_2$-GLOBULIN LEVELS (DAY O - DAY X) OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.52	0.67	0.77	>0.50	N.S.
2	- 1.15	0.65	1.77	>0.10	N.S.
3	- 0.05	0.42	0.12	>0.90	N.S.
4	+ 0.68	0.68	1.00	>0.40	N.S.
5	- 0.96	0.74	1.30	>0.30	N.S.
6	- 0.60	0.63	0.95	>0.40	N.S.
7	- 1.08	0.61	1.76	>0.10	N.S.
8	+ O.O3	0.66	0.05	>0.90	N.S.
9	- O.45	0.59	0.76	>0.50	N.S.
10	- 0.58	0.67	0.87	>0.40	N.S.
11	- 0.89	0.59	1.51	>0.20	N.S.
12	- 1.38	0.74	1.86	>0.10	N.S.
13	- 0.56	0.70	0.81	>0.50	N.S.
14	- 0.46	0.67	0.69	>0.50	N.S.
21	- 0.99	0.64	1.55	>0.20	N.S.
28	- 0.46	0.73	0.63	>0.60	N.S.
35	- 0.53	0.55	1.OO	>0.40	N.S.

N.S. = Not Significant.

TABLE 5.

MEAN DIFFERENCE OF BETA-GLOBULIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.56	0.69	0.81	> 0.50	N.S.
2	- 0.57	0.98	0.58	> 0.60	N.S.
3	- 0.41	0.73	0.56	> 0.60	N.S.
4	+ 0.05	0.45	0.11	> 0.90	N.S.
5	- 1.59	0.88	1.81	> 0.10	N.S.
6	+ 0.08	0.74	0.10	> 0.90	N.S.
7	- 0.03	0.62	0.04	> 0.90	N.S.
8	+ 0.16	0.72	0.22	> 0.90	N.S.
9	- 0.53	0.61	0.86	> 0.50	N.S.
10	- 0.32	0.64	0.50	> 0.60	N.S.
11	- 0.75	0.62	1.21	> 0.30	N.S.
12	- 0.01	0.56	0.01	> 0.90	N.S.
13	+ 0.25	0.53	0.47	> 0.70	N.S.
14	- 0.13	0.64	0.24	> 0.90	N.S.
21	- 0.13	0.56	0.22	> 0.90	N.S.
28	- 0.11	0.50	0.21	> 0.90	N.S.
35	+ 0.70	0.41	2.43	< 0.05	S.

S. = Significant.

N.S. = Not Significant.

TABLE 6.

MEAN DIFFERENCE OF GAMMAGLOBULIN LEVELS (DAY 0 - DAY X) OF SHEEP REINFECTED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 1.49	0.69	2.14	$<$ 0.05	S.
2	- 0.77	1.54	0.50	$>$ 0.50	N.S.
3	- 0.68	0.82	0.82	$>$ 0.50	N.S.
4	+ 1.71	1.30	1.31	$>$ 0.30	N.S.
5	- 0.13	1.30	0.64	$>$ 0.60	N.S.
6	- 0.13	0.86	0.15	$>$ 0.90	N.S.
7	- 1.53	1.18	1.30	$>$ 0.30	N.S.
8	- 2.10	1.06	1.98	$>$ 0.10	N.S.
9	- 4.43	1.36	3.26	$<$ 0.01	H.S.
10	- 3.16	1.23	2.57	$<$ 0.02	S.
11	- 4.00	1.21	3.31	$<$ 0.01	H.S.
12	- 4.05	1.07	3.74	$<$ 0.01	H.S.
13	- 2.99	1.10	2.72	$<$ 0.02	S.
14	- 2.37	0.92	2.58	$<$ 0.02	S.
21	- 0.80	1.09	0.73	$>$ 0.50	N.S.
28	+ 0.51	1.08	0.47	$>$ 0.70	N.S.
35	- 0.77	1.18	0.65	$>$ 0.60	N.S.

S = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 7

MEAN DIFFERENCE OF TOTAL SERUM PROTEIN CONTENTS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.80	1.59	0.50	> 0.70	N.S.
2	+ 4.20	1.07	3.92	< 0.02	S.
3	+ 1.40	0.24	5.80	< 0.01	H.S.
4	+ 4.60	1.29	3.56	< 0.05	S.
5	+ 5.60	1.43	3.92	< 0.02	S.
6	+ 0.80	1.65	0.48	> 0.70	N.S.
7	+ 0.80	0.97	0.82	> 0.50	N.S.
8	+ 2.40	1.03	2.33	> 0.10	N.S.
9	+ 2.60	1.50	1.73	> 0.20	N.S.
10	+ 3.00	2.68	1.12	> 0.40	N.S.
11	+ 2.80	1.32	2.12	> 0.10	N.S.
12	+ 4.20	1.46	2.87	< 0.05	S.
13	+ 2.40	2.33	1.03	> 0.40	N.S.
14	+ 1.60	0.60	2.66	< 0.05	S.
21	+ 1.20	0.20	6.00	< 0.01	H.S.
28	+ 0.20	0.58	0.34	> 0.80	N.S.
35	+ 1.80	1.11	1.62	> 0.20	N.S.

S = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 8.

MEAN DIFFERENCE OF ALBUMIN LEVELS (DAY 0 - DAY X) OF UN-CHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 2.30	2.53	0.91	> 0.50	N.S.
2	- 0.60	2.72	0.22	> 0.90	N.S.
3	+ 1.02	3.21	0.32	> 0.80	N.S.
4	+ 0.96	3.11	0.31	> 0.80	N.S.
5	+ 5.26	3.24	1.62	> 0.20	N.S.
6	- 1.48	2.62	0.56	> 0.60	N.S.
7	- 2.36	2.58	0.91	> 0.50	N.S.
8	+ 0.38	4.36	0.09	> 0.90	N.S.
9	- 0.14	2.91	0.05	> 0.90	N.S.
10	+ 1.18	3.67	0.32	> 0.80	N.S.
11	+ 1.26	3.25	0.39	> 0.80	N.S.
12	+ 0.60	4.00	0.15	> 0.90	N.S.
13	- 1.36	3.99	0.34	> 0.80	N.S.
14	- 2.50	4.05	0.62	> 0.60	N.S.
21	- 1.06	3.57	0.30	> 0.80	N.S.
28	- 2.30	3.29	0.70	> 0.60	N.S.
35	- 4.00	3.24	1.23	> 0.30	N.S.

N.S. = Not Significant.

TABLE 9.

MEAN DIFFERENCE OF ALPHA$_1$-GLOBULIN LEVELS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.36	0.44	0.82	> 0.50	N.S.
2	+ 0.52	0.82	0.63	> 0.60	N.S.
3	+ 0.32	0.38	0.84	> 0.50	N.S.
4	+ 0.08	0.22	0.36	> 0.80	N.S.
5	- 0.86	0.91	0.95	> 0.40	N.S.
6	+ 0.34	0.31	1.10	> 0.40	N.S.
7	+ 0.46	0.47	0.98	> 0.40	N.S.
8	+ 0.04	0.22	0.18	> 0.90	N.S.
9	+ 0.36	0.36	1.00	> 0.40	N.S.
10	+ 0.36	0.27	1.33	> 0.30	N.S.
11	- 0.03	0.19	0.16	> 0.90	N.S.
12	- 0.64	0.56	1.14	> 0.40	N.S.
13	+ 0.12	0.65	0.18	> 0.90	N.S.
14	+ 0.04	0.40	0.10	> 0.90	N.S.
21	+ 0.32	0.33	0.97	> 0.40	N.S.
28	- 0.06	0.64	0.09	> 0.90	N.S.
35	+ 0.61	0.91	0.67	> 0.60	N.S.

N.S. = Not Significant.

TABLE 10.

MEAN DIFFERENCE OF ALPHA$_2$-GLOBULIN LEVELS (DAY 0 - DAY X) OF UN-CHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 2.14	2.50	0.86	>0.50	N.S.
2	+ 0.18	1.18	0.15	>0.90	N.S.
3	+ 0.06	0.57	0.11	>0.90	N.S.
4	+ 0.32	0.60	0.53	>0.70	N.S.
5	- 0.12	0.87	0.14	>0.90	N.S.
6	- 0.02	1.03	0.02	>0.90	N.S.
7	+ 0.48	0.85	0.56	>0.60	N.S.
8	+ 0.76	1.23	0.62	>0.60	N.S.
9	+ 0.76	0.86	0.88	>0.50	N.S.
10	+ 0.80	0.93	0.86	>0.50	N.S.
11	- 0.10	0.71	0.14	>0.90	N.S.
12	+ 0.04	0.90	0.04	>0.90	N.S.
13	- 0.44	0.84	0.52	>0.70	N.S.
14	+ 0.74	1.02	0.73	>0.50	N.S.
21	+ 0.28	0.86	0.32	>0.80	N.S.
28	- 0.22	0.30	0.73	>0.50	N.S.
35	+ 0.46	0.82	0.56	>0.70	N.S.

N.S. = Not Significant.

TABLE 11.

MEAN DIFFERENCES OF BETA-GLOBULIN LEVELS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenged (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.18	0.33	0.54	>0.70	N.S.
2	- 0.42	0.84	0.50	>0.70	N.S.
3	+ 0.54	0.84	0.64	>0.60	N.S.
4	- 0.52	1.12	0.46	>0.70	N.S.
5	- 1.22	0.75	1.62	>0.20	N.S.
6	- 0.02	0.31	0.06	>0.90	N.S.
7	- 0.36	0.44	0.82	>0.50	N.S.
8	+ 0.38	0.33	1.15	>0.40	N.S.
9	- 0.16	0.36	0.44	>0.70	N.S.
10	+ 0.20	0.75	0.27	>0.80	N.S.
11	- 0.08	0.52	0.15	>0.90	N.S.
12	- 0.52	1.04	0.50	>0.70	N.S.
13	+ 0.02	1.07	0.02	>0.90	N.S.
14	+ 0.42	0.60	0.70	>0.60	N.S.
21	- 0.36	0.72	0.50	>0.70	N.S.
28	- 0.88	0.44	2.00	>0.20	N.S.
35	- 0.46	0.50	0.92	>0.40	N.S.

N.S. = Not Significant.

TABLE 12.

MEAN DIFFERENCE OF GAMMA-GLOBULIN LEVELS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.02	0.90	0.02	> 0.90	N.S.
2	+ 0.80	1.08	0.74	> 0.50	N.S.
3	+ 0.44	0.72	0.61	> 0.60	N.S.
4	+ 0.64	0.63	1.02	> 0.40	N.S.
5	+ 0.00	0.75	0.00	> 0.90	N.S.
6	+ 0.80	1.16	0.69	> 0.60	N.S.
7	+ 1.16	1.18	0.98	> 0.40	N.S.
8	- 0.74	1.04	0.71	> 0.60	N.S.
9	+ 0.54	0.55	0.98	> 0.40	N.S.
10	- 0.18	0.73	0.25	> 0.90	N.S.
11	+ 0.66	0.74	0.89	> 0.50	N.S.
12	+ 1.20	2.03	0.59	> 0.60	N.S.
13	+ 1.06	2.17	0.49	> 0.70	N.S.
14	+ 0.82	0.97	0.84	> 0.50	N.S.
21	+ 1.00	1.58	0.63	> 0.60	N.S.
28	+ 1.06	0.96	1.10	> 0.40	N.S.
35	+ 1.16	1.55	0.75	> 0.50	N.S.

N.S. = Not Significant.

TABLE 13.

COMPARISON OF THE DAILY MEANS GAMMAGLOBULIN LEVELS BETWEEN GROUPS 1 AND 2.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	0.35	> 0.80	N.S.
1	21	0.15	> 0.90	N.S.
2	17	0.81	> 0.50	N.S.
3	17	0.38	> 0.80	N.S.
4	12	1.13	> 0.30	N.S.
5	17	0.33	> 0.80	N.S.
6	17	0.32	> 0.80	N.S.
7	17	0.68	> 0.60	N.S.
8	17	0.69	> 0.50	N.S.
9	21	1.48	> 0.20	N.S.
10	21	1.08	> 0.30	N.S.
11	21	1.53	> 0.20	N.S.
12	17	2.23	< 0.05	S.
13	21	2.31	< 0.05	S.
14	21	2.12	< 0.05	S.
21	21	1.46	> 0.20	N.S.
28	21	0.75	> 0.50	N.S.
35	21	2.04	> 0.10	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 14.

MEAN DIFFERENCE OF TOTAL SERUM PROTEIN CONTENTS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (X)	Mean difference	Standard Error	t	p	Inter-pretation
1	+ 1.87	1.31	1.43	> 0.20	N.S.
3	+ 4.87	1.45	3.36	< 0.01	H.S.
5	+ 1.87	1.49	1.26	> 0.30	N.S.
7	+ 4.38	1.68	2.60	< 0.05	S.
8	+ 0.38	2.43	0.15	> 0.90	N.S.
9	+ 2.38	2.53	0.94	> 0.40	N.S.
10	+ 0.75	2.56	0.29	> 0.80	N.S.
11	+ 0.38	1.73	0.22	> 0.90	N.S.
12	+ 1.50	2.08	0.72	> 0.50	N.S.
13	- 0.86	2.46	0.35	> 0.80	N.S.
14	+ 2.14	2.43	0.88	> 0.50	N.S.
21	- 0.71	3.18	0.22	> 0.90	N.S.
28	+ 0.86	1.94	0.44	> 0.70	N.S.
35	+ 3.00	1.78	1.68	> 0.20	N.S.

H.S. = Highly Significant.

N.S. = Not Significant.

S. = Significant.

TABLE 15.

MEAN DIFFERENCE OF ALBUMIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	p	Interpretation
1	- 0.95	1.19	0.80	> 0.50	N.S.
3	+ 3.19	1.61	1.98	> 0.10	N.S.
5	+ 2.04	1.74	1.17	> 0.30	N.S.
6	+ 3.05	0.92	3.31	< 0.02	S.
7	+ 3.38	1.10	3.08	< 0.02	S.
8	+ 1.36	0.79	1.72	> 0.20	N.S.
9	+ 0.50	1.27	0.39	> 0.80	N.S.
10	+ 1.56	1.24	1.26	> 0.30	N.S.
11	+ 0.26	1.05	0.25	> 0.90	N.S.
12	+ 2.44	0.73	3.34	< 0.02	S.
13	+ 3.40	1.29	2.71	< 0.05	S.
14	+ 0.83	1.30	0.64	> 0.60	N.S.
21	- 0.83	1.28	0.65	> 0.60	N.S.
28	+ 0.16	1.11	0.14	> 0.90	N.S.
35	+ 1.58	1.36	1.16	> 0.30	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 16.

MEAN DIFFERENCE OF ALPHA$_1$-GLOBULIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	p	Interpretation
1	0.00	0.27	0.00	> 0.90	N.S.
3	- 0.55	0.36	1.53	> 0.20	N.S.
5	- 0.75	0.46	1.63	> 0.20	N.S.
6	- 0.46	0.33	1.40	> 0.20	N.S.
7	- 0.82	0.40	2.05	> 0.10	N.S.
8	- 0.86	0.40	2.16	> 0.10	N.S.
9	- 0.34	0.47	0.72	> 0.50	N.S.
10	- 0.31	0.36	0.86	> 0.50	N.S.
11	- 0.62	0.27	2.31	> 0.10	N.S.
12	- 0.39	0.38	1.01	> 0.40	N.S.
13	- 0.77	0.33	2.34	> 0.10	N.S.
14	- 0.41	0.29	1.43	> 0.20	N.S.
21	- 0.24	0.47	0.52	> 0.70	N.S.
28	+ 0.03	0.46	0.06	> 0.90	N.S.
35	- 0.14	0.55	0.26	> 0.90	N.S.

N.S. = Not Significant.

TABLE 17.

MEAN DIFFERENCE OF ALPHA$_2$-GLOBULIN LEVELS (DAY O - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ O.63	0.33	1.89	>0.20	N.S.
3	- O.1O	0.48	0.59	>0.60	N.S.
5	- 0.45	0.58	0.78	>0.50	N.S.
6	- 0.25	0.52	0.48	>0.70	N.S.
7	- 0.10	0.52	0.19	>0.90	N.S.
8	- 0.58	0.89	0.65	>0.60	N.S.
9	- 0.60	0.60	1.00	>0.40	N.S.
1O	- 0.41	0.33	1.25	>0.30	N.S.
11	- 0.38	0.42	0.89	>0.50	N.S.
12	- 0.64	0.48	1.34	>0.30	N.S.
13	+ O.11	0.70	0.16	>0.90	N.S.
14	- 0.13	0.34	0.38	>0.80	N.S.
21	- 0.53	0.78	0.68	>0.60	N.S.
28	+ O.O1	O.34	O.O4	>0.90	N.S.
35	- 0.77	0.39	1.98	>0.10	N.S.

N.S. = Not Significant.

TABLE 18.

MEAN DIFFERENCE OF BETA-GLOBULIN LEVELS (DAY O - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	p	Inter-pretation
1	+ 0.61	0.39	1.57	>0.20	N.S.
3	- 0.31	0.51	0.61	>0.60	N.S.
5	+ 0.25	0.47	0.53	>0.70	N.S.
6	+ 0.74	0.42	1.76	>0.20	N.S.
7	+ 0.64	0.65	0.98	>0.40	N.S.
8	+ 0.23	0.76	0.30	>0.80	N.S.
9	+ 0.95	0.60	1.58	>0.20	N.S.
10	+ 0.36	0.67	0.54	>0.70	N.S.
11	+ 0.59	0.58	1.01	>0.40	N.S.
12	- 0.16	0.80	0.20	>0.90	N.S.
13	- 0.18	0.69	0.27	>0.80	N.S.
14	+ 1.01	0.58	1.75	>0.20	N.S.
21	+ 0.40	0.93	0.43	>0.70	N.S.
28	+ 1.00	0.69	1.45	>0.20	N.S.
35	+ 0.34	0.62	0.55	>0.60	N.S.

N.S. = Not Significant.

TABLE 19.

MEAN DIFFERENCE OF GAMMAGLOBULIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post Challenge (X)	Mean Difference	Standard Error	t	p	Inter-pretation
1	+ 1.50	0.80	1.88	> 0.20	N.S.
3	+ 1.80	1.10	1.64	> 0.20	N.S.
5	+ 0.70	1.30	0.54	> 0.70	N.S.
6	+ 0.65	2.50	0.26	> 0.90	N.S.
7	+ 0.45	1.00	0.45	> 0.70	N.S.
8	+ 0.80	1.20	0.67	> 0.60	N.S.
9	+ 0.23	1.50	0.15	> 0.90	N.S.
10	+ 0.80	1.40	0.57	> 0.60	N.S.
11	+ 1.60	1.20	1.33	> 0.30	N.S.
12	+ 0.60	1.50	0.40	> 0.80	N.S.
13	+ 2.50	1.50	1.67	> 0.20	N.S.
14	+ 1.70	1.30	1.31	> 0.20	N.S.
21	+ 1.40	1.40	1.00	> 0.40	N.S.
28	+ 0.90	1.10	0.82	> 0.50	N.S.
35	+ 0.20	1.30	0.15	> 0.90	N.S.

N.S. = Not Significant

TABLE 20.

COMPARISON OF THE DAILY MEANS OF GAMMAGLOBULIN LEVELS/BETWEEN GROUPS 1 AND 3.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	0.45	>0.70	N.S.
1	21	0.33	>0.80	N.S.
3	17	0.08	>0.90	N.S.
5	17	0.38	>0.80	N.S.
6	14	0.56	>0.60	N.S.
7	17	0.39	>0.80	N.S.
8	15	0.11	>0.90	N.S.
9	21	0.62	>0.60	N.S.
10	21	0.08	>0.90	N.S.
11	21	0.32	>0.80	N.S.
12	17	0.16	>0.90	N.S.
13	21	0.03	>0.90	N.S.
14	21	0.04	>0.90	N.S.
21	21	0.77	>0.50	N.S.
28	21	1.19	>0.30	N.S.
35	21	1.08	>0.30	N.S.

N.S. = Not Significant.

TABLE 21.

MEAN DIFFERENCE OF IgM LEVELS (DAY O - DAY X) OF SHEEP PRE-INFECTED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.28	0.19	1.45	>0.20	N.S.
2	- 0.88	0.31	2.84	<0.02	S.
3	- 0.87	0.46	1.86	>0.10	N.S.
4	- 0.95	0.56	1.70	>0.20	N.S.
5	- 0.55	0.28	1.96	>0.10	N.S.
6	- 0.39	0.27	1.44	>0.10	N.S.
7	+ 0.08	0.33	0.23	>0.90	N.S.
8	+ 0.20	0.23	0.87	>0.50	N.S.
9	+ 0.01	0.21	0.06	>0.90	N.S.
10	+ 0.46	0.29	1.59	>0.20	N.S.
11	- 0.17	0.23	0.74	>0.50	N.S.
12	- 0.70	0.33	2.12	>0.10	N.S.
13	- 0.36	0.21	1.71	>0.20	N.S.
14	- 0.33	0.22	1.50	>0.20	N.S.
21	- 0.09	0.22	0.41	>0.70	N.S.
28	- 0.13	0.20	0.65	>0.60	N.S.
35	- 0.07	0.19	0.37	>0.80	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 22.

MEAN DIFFERENCE IN IgG_1 LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.98	0.93	1.05	> 0.40	N.S.
2	- 0.44	0.98	0.45	> 0.70	N.S.
3	+ 0.96	1.01	0.95	> 0.40	N.S.
4	-2.48	1.52	1.63	> 0.20	N.S.
5	+ 0.74	1.16	0.64	> 0.30	N.S.
6	- 1.42	1.68	0.85	> 0.50	N.S.
7	- 1.77	1.62	1.09	> 0.30	N.S.
8	- 2.27	1.91	1.19	> 0.30	N.S.
9	- 3.07	1.66	1.85	> 0.20	N.S.
10	- 3.53	1.77	1.99	> 0.10	N.S.
11	- 4.83	1.67	2.89	< 0.02	S.
12	- 5.81	1.81	3.21	< 0.01	H.S.
13	- 5.21	1.77	2.94	< 0.01	H.S.
14	- 6.27	1.89	3.32	< 0.01	H.S.
21	- 4.14	1.67	2.48	< 0.02	S.
28	- 4.61	1.35	3.41	< 0.01	H.S.
35	- 3.28	1.37	2.39	< 0.05	S.

S. = Significant.

H. S. = Highly Significant.

N. S. = Not Significant.

TABLE 23.

MEAN DIFFERENCES OF IgG_2 LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP I.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.2	0.18	0.02	> 0.90	N.S.
2	- 0.51	0.29	1.76	> 0.10	N.S.
3	- 0.55	0.30	1.83	> 0.10	N.S.
4	- 0.625	0.40	1.56	> 0.20	N.S.
5	- 0.575	0.30	1.92	> 0.10	N.S.
6	- 0.625	0.82	0.75	> 0.50	N.S.
7	- 0.43	0.26	1.65	> 0.20	N.S.
8	- 0.13	0.40	0.32	> 0.80	N.S.
9	- 0.62	0.36	1.76	> 0.50	N.S.
10	- 0.675	0.51	1.32	> 0.30	N.S.
11	- 0.92	0.44	2.09	> 0.10	N.S.
12	- 0.83	0.31	2.68	< 0.05	S.
13	- 0.71	0.37	1.92	> 0.10	N.S.
14	- 0.46	0.42	1.10	> 0.30	N.S.
21	- 0.27	0.34	0.79	> 0.50	N.S.
28	- 0.34	0.39	0.87	> 0.40	N.S.
35	0.07	0.32	0.22	> 0.90	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 24.

MEAN DIFFERENCE OF IgM LEVELS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.20	0.08	2.50	> 0.10	N.S.
2	+ 0.12	0.20	0.60	> 0.60	N.S.
3	+ 0.06	0.22	0.27	> 0.80	N.S.
4	- 0.12	0.15	0.80	> 0.50	N.S.
5	+ 0.06	0.30	0.20	> 0.90	N.S.
6	+ 0.06	0.22	0.27	> 0.80	N.S.
7	- 0.06	0.29	0.21	> 0.90	N.S.
8	- 0.24	0.20	1.20	> 0.30	N.S.
9	- 0.20	0.25	0.80	> 0.60	N.S.
10	- 0.38	0.24	1.58	> 0.20	N.S.
11	- 0.30	0.27	1.11	> 0.40	N.S.
12	- 0.20	0.25	0.25	> 0.90	N.S.
13	- 0.36	0.17	2.11	> 0.10	N.S.
14	- 0.36	0.31	1.16	> 0.30	N.S.
21	- 0.18	0.26	0.70	> 0.60	N.S.
28	- 0.24	0.26	0.93	> 0.40	N.S.
35	+ 0.02	0.16	0.12	> 0.90	N.S.

Table 25.

MEAN DIFFERENCE OF IgG_1 LEVELS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	0.00	0.56	0.00	>0.90	N.S.
2	0.00	0.50	0.00	>0.90	N.S.
3	+ 0.32	0.32	1.00	>0.40	N.S.
4	0.00	0.36	0.00	>0.90	N.S.
5	- 0.32	0.65	0.49	>0.70	N.S.
6	0.00	0.25	0.00	>0.90	N.S.
7	+ 0.14	0.46	0.30	>0.80	N.S.
8	+ 0.32	0.54	0.59	>0.60	N.S.
9	- 0.18	0.46	0.39	>0.80	N.S.
10	+ 0.14	0.80	0.17	>0.90	N.S.
11	- 0.32	0.65	0.49	>0.70	N.S.
12	0.00	0.51	0.00	>0.90	N.S.
13	+ 0.48	0.41	1.17	>0.40	N.S.
14	+ 0.16	0.30	0.53	>0.70	N.S.
21	+ 0.64	0.39	1.64	>0.20	N.S.
28	+ 0.64	0.39	1.64	>0.20	N.S.
35	+ 0.16	0.46	0.35	>0.80	N.S.

N.S. = Not Significant.

Table 26.

MEAN DIFFERENCE OF IgG_2 LEVELS (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.27	0.15	1.83	> 0.20	N.S.
2	+ 0.04	0.10	0.40	> 0.80	N.S.
3	+ 0.10	0.11	0.91	> 0.50	N.S.
4	+ 0.08	0.12	0.67	> 0.60	N.S.
5	+ 0.00	0.19	0.00	> 0.90	N.S.
6	- 0.24	0.14	1.71	> 0.20	N.S.
7	- 0.02	0.97	0.21	> 0.90	N.S.
8	+ 0.00	0.19	0.00	> 0.90	N.S.
9	+ 0.02	0.11	0.18	> 0.90	N.S.
10	- 0.02	0.10	0.20	> 0.90	N.S.
11	- 0.22	0.24	0.92	> 0.40	N.S.
12	- 0.22	0.09	2.40	> 0.10	N.S.
13	- 0.22	0.14	1.57	> 0.20	N.S.
14	+ 0.08	0.27	0.30	> 0.80	N.S.
21	+ 0.08	0.27	0.30	> 0.80	N.S.
28	+ 0.20	0.22	0.91	> 0.50	N.S.
35	- 0.14	0.14	1.00	> 0.40	N.S.

N.S. = Not Significant.

TABLE 27.

COMPARISON OF THE DAILY MEANS OF IgG_1 BETWEEN GROUPS 1 AND 2.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	1.03	> 0.40	N.S.
1	21	0.79	> 0.50	N.S.
2	17	1.54	> 0.20	N.S.
3	17	1.07	> 0.30	N.S.
4	13	2.87	< 0.05	S.
5	17	0.82	> 0.50	N.S.
6	17	1.86	> 0.10	N.S.
7	17	1.62	> 0.20	N.S.
8	17	2.29	< 0.05	S.
9	21	1.93	> 0.10	N.S.
10	21	2.36	< 0.05	S.
11	21	2.20	< 0.05	S.
12	17	2.29	< 0.05	S.
13	21	2.18	< 0.05	S.
14	21	2.66	< 0.02	S.
21	21	2.30	< 0.05	S.
28	21	3.22	< 0.01	H.S.
35	21	2.72	< 0.02	S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 28.

COMPARISON OF THE DAILY MEANS OF IgM LEVELS BETWEEN GROUPS 1 AND 2

Days Post Challenge	N	t	P	Inter-pretation
0	21	0.43	> 0.60	N.S.
1	21	0.83	> 0.50	N.S.
2	17	1.63	> 0.20	N.S.
3	17	1.55	> 0.20	N.S.
4	13	0.99	> 0.40	N.S.
5	17	1.51	> 0.20	N.S.
6	17	0.97	> 0.40	N.S.
7	17	0.28	> 0.80	N.S.
8	17	0.37	> 0.80	N.S.
9	21	0.03	> 0.90	N.S.
10	21	0.28	> 0.80	N.S.
11	21	0.10	> 0.90	N.S.
12	17	1.18	> 0.30	N.S.
13	21	0.33	> 0.80	N.S.
14	21	0.32	> 0.80	N.S.
21	21	0.21	> 0.90	N.S.
28	21	0.08	> 0.90	N.S.
35	21	0.52	> 0.70	N.S.

N.S. = Not Significant.

TABLE 29.

COMPARISON OF THE DAILY MEANS OF IgG_2 LEVELS BETWEEN GROUPS 1 AND 2.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	0.77	>0.50	N.S.
1	21	0.84	>0.50	N.S.
2	17	0.93	>0.40	N.S.
3	17	0.99	>0.40	N.S.
4	13	2.06	>0.10	N.S.
5	17	0.86	>0.40	N.S.
6	17	1.05	>0.40	N.S.
7	17	0.80	>0.50	N.S.
8	17	0.66	>0.60	N.S.
9	21	0.99	>0.40	N.S.
10	21	1.09	>0.30	N.S.
11	21	1.15	>0.30	N.S.
12	17	0.90	>0.40	N.S.
13	21	1.12	>0.30	N.S.
14	21	1.19	>0.30	N.S.
21	21	1.18	>0.30	N.S.
28	21	1.25	>0.30	N.S.
35	21	0.75	>0.50	N.S.

N.S. = Not Significant.

TABLE 30.

MEAN DIFFERENCE OF IgM LEVELS (DAY O - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (x)	Mean difference	Standard Error	t	p	Interpretation
1	0.00	0.16	0.00	>0.90	N.S.
3	+ 0.41	0.24	1.72	>0.20	N.S.
5	+ 0.06	0.20	0.31	>0.80	N.S.
6	+ 0.18	0.33	0.53	>0.70	N.S.
7	+ 0.15	0.25	0.60	>0.60	N.S.
8	+ 0.18	0.32	0.57	>0.60	N.S.
9	- 0.88	0.45	0.19	>0.90	N.S.
10	- 0.23	0.37	0.61	>0.60	N.S.
11	+ 0.16	0.25	0.65	>0.60	N.S.
12	+ 0.10	0.34	0.29	>0.80	N.S.
13	+ 0.10	0.34	0.29	>0.80	N.S.
14	+ 0.06	0.30	0.19	>0.90	N.S.
21	+ 0.20	0.25	0.80	>0.50	N.S.
28	+ 0.20	0.21	0.95	>0.40	N.S.
35	+ 0.17	0.21	0.82	>0.50	N.S.

N.S. = Not Significant.

TABLE 31.

MEAN DIFFERENCE OF IgG_1 LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (x)	Mean difference	Standard Error	t	p	Inter-pretation
1	+ 0.29	0.35	0.83	> 0.50	N.S.
3	+ 0.58	2.38	2.38	< 0.05	S.
5	+ 0.36	0.72	0.49	> 0.70	N.S.
6	- 0.28	1.67	0.16	> 0.90	N.S.
7	- 0.48	0.69	0.69	> 0.60	N.S.
8	- 1.83	1.05	1.74	> 0.20	N.S.
9	- 1.39	0.73	1.90	> 0.10	N.S.
10	- 2.12	0.99	2.15	> 0.10	N.S.
11	- 1.91	0.81	2.36	< 0.05	S.
12	- 1.18	0.58	2.03	> 0.10	N.S.
13	- 1.17	1.05	1.11	> 0.40	N.S.
14	- 1.64	0.89	1.84	> 0.20	N.S.
21	- 1.73	0.85	2.03	> 0.10	N.S.
28	- 1.00	0.81	1.18	> 0.30	N.S.
35	- 0.11	1.39	0.08	> 0.90	N.S.

S. = significant

N.S. = Not significant

TABLE 32.

MEAN DIFFERENCE OF IgG_2 LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.08	0.20	0.38	> 0.80	N.S.
3	- 0.40	0.28	1.45	> 0.20	N.S.
5	- 0.23	0.27	0.83	> 0.50	N.S.
6	- 0.68	0.31	2.18	> 0.10	N.S.
7	- 0.31	0.21	1.49	> 0.20	N.S.
8	- 0.32	0.18	1.76	> 0.20	N.S.
9	- 0.81	0.30	2.71	< 0.05	S
10	- 0.71	0.25	2.85	< 0.05	S
11	- 0.70	0.35	2.00	> 0.10	N.S.
12	- 0.51	0.40	1.28	> 0.30	N.S.
13	- 0.60	0.41	1.46	> 0.20	N.S.
14	- 0.70	0.31	2.90	< 0.05	S
21	- 0.91	0.38	2.41	< 0.05	S
28	- 0.69	0.30	2.29	> 0.10	N.S.
35	- 0.86	0.25	3.43	< 0.02	S

S = Significant

N.S. = Not significant

TABLE 33.

COMPARISON OF THE DAILY MEANS OF IgG_1 LEVELS BETWEEN GROUPS 1 AND 3.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	1.34	>0.20	N.S.
1	21	1.06	>0.30	N.S.
3	17	0.96	>0.40	N.S.
5	17	1.34	>0.20	N.S.
6	14	1.43	>0.20	N.S.
7	17	1.78	>0.10	N.S.
8	15	2.71	<0.02	S.
9	21	1.93	>0.10	N.S.
10	21	1.84	>0.10	N.S.
11	17	1.99	>0.10	N.S.
12	17	2.47	<0.05	S.
13	21	2.27	<0.05	S.
14	21	2.73	<0.02	S.
21	21	2.10	<0.05	S.
28	21	3.34	<0.01	H.S.
35	21	3.11	<0.01	H.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 34.

COMPARISON OF THE DAILY MEANS OF IgM LEVELS BETWEEN GROUPS 1 and 3.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	0.68	>0.60	N.S.
1	21	1.32	>0.30	N.S.
3	17	2.43	>0.05	S.
5	17	2.10	>0.05	S.
6	14	0.88	>0.40	N.S.
7	17	1.24	>0.30	N.S.
8	15	1.17	>0.30	N.S.
9	21	1.02	>0.40	N.S.
10	21	1.54	>0.20	N.S.
11	21	1.51	>0.20	N.S.
12	17	2.17	>0.05	S.
13	21	1.56	>0.20	N.S.
14	21	1.24	>0.30	N.S.
21	21	1.85	>0.10	N.S.
28	21	0.78	>0.50	N.S.
35	21	1.15	>0.30	N.S.

N.S. = Not Significant.

TABLE 35.

COMPARISON OF THE DAILY MEANS OF IgG_2 LEVELS BETWEEN GROUPS 1 AND 3.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	1.14	>0.30	N.S.
1	21	1.09	>0.30	N.S.
3	17	1.08	>0.30	N.S.
5	17	1.06	>0.40	N.S.
6	17	1.21	>0.30	N.S.
7	17	1.01	>0.40	N.S.
8	15	1.25	>0.30	N.S.
9	21	0.82	>0.50	N.S.
10	21	1.04	>0.40	N.S.
11	21	1.24	>0.30	N.S.
12	17	1.15	>0.30	N.S.
13	21	1.28	>0.30	N.S.
14	21	0.84	>0.50	N.S.
21	21	0.88	>0.40	N.S.
28	21	0.95	>0.40	N.S.
35	21	1.19	>0.30	N.S.

N.S. = Not Signficant.

TABLE 36.

COMPARISON OF DAILY MEANS OF ORF ANTIBODY TITRES BETWEEN GROUPS 1 AND 3.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	21	2.62	< 0.050	S.
1	21	2.62	< 0.050	S.
3	17	3.53	< 0.010	H.S.
5	17	5.20	< 0.001	H.S.
6	17	1.99	> 0.100	N.S.
7	17	5.59	< 0.001	H.S.
8	17	4.70	< 0.001	H.S.
9	21	3.85	< 0.001	H.S.
10	21	3.70	< 0.010	H.S.
11	21	3.86	< 0.001	H.S.
12	17	3.97	< 0.001	H.S.
13	21	4.39	< 0.001	H.S.
14	21	4.62	< 0.001	H.S.
21	21	3.06	< 0.010	H.S.
28	21	2.23	< 0.050	S.
35	21	2.23	< 0.050	S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 37.

MEAN DIFFERENCES IN ANTIBODY TITRES (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 1.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	0.00	0.00	0.00	> 0.900	N.S.
2	0.00	0.00	0.00	> 0.900	N.S.
3	- 0.25	0.13	1.92	> 0.100	N.S.
4	- 0.25	0.13	1.92	> 0.100	N.S.
5	- 0.58	0.22	2.64	< 0.050	S.
6	- 0.50	0.19	2.63	< 0.050	S.
7	- 1.16	0.27	4.30	< 0.010	H.S.
8	- 1.83	0.23	7.96	< 0.001	H.S.
9	- 1.81	0.26	6.96	< 0.001	H.S.
10	- 1.94	0.26	7.32	< 0.001	H.S.
11	- 2.25	0.21	10.40	< 0.001	H.S.
12	- 2.25	0.35	6.40	< 0.001	H.S.
13	- 2.37	0.27	8.80	< 0.001	H.S.
14	- 2.25	0.29	7.63	< 0.001	H.S.
21	- 1.94	0.25	7.76	< 0.001	H.S.
28	- 1.44	0.24	6.00	< 0.001	H.S.
35	- 1.37	0.20	6.85	< 0.001	H.S.

S. = Significant

H.S. = Highly Significant

N.S. = Not Significant

TABLE 39.

CORRELATION BETWEEN THE GAMMAGLOBULIN LEVELS AND ANTIBODY TITRES OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP I.

Number of Animal	Correlation Coefficient (r)	P	Inter-pretation
751	+ 0.11	> 0.05	N.S.
780	+ 0.05	> 0.05	N.S.
749	+ 0.55	< 0.05	S.
765	+ 0.50	< 0.05	S.
767	+ 0.57	< 0.05	S.
779	+ 0.45	> 0.05	N.S.
768	+ 0.60	< 0.01	H.S.
691	- 0.01	> 0.05	N.S.
740	+ 0.32	> 0.05	N.S.
712	- 0.18	> 0.05	N.S.
790	+ 0.70	< 0.01	H.S.
362	+ 0.14	> 0.05	N.S.
852	- 0.31	> 0.05	N.S.
875	+ 0.59	< 0.05	S.
836	+ 0.85	< 0.01	H.S.
838	- 0.15	> 0.05	N.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 41.

CORRELATION BETWEEN THE IgG_1 LEVELS AND ANTIBODY TITRES OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP I.

Number of Animal	Correlation Coefficient (r)	P	Inter-pretation
751	- 0.62	< 0.01	H.S.
780	+ 0.32	> 0.05	N.S.
749	+ 0.11	> 0.05	N.S.
765	+ 0.81	< 0.01	H.S.
767	+ 0.74	< 0.01	H.S.
779	+ 0.86	< 0.01	H.S.
768	+ 0.82	< 0.01	H.S.
691	+ 0.62	< 0.01	H.S.
740	+ 0.32	> 0.05	N.S.
712	+ 0.53	< 0.05	S.
790	+ 0.74	< 0.01	H.S.
362	+ 0.30	> 0.05	N.S.
852	+ 0.62	< 0.05	S.
875	+ 0.09	> 0.05	N.S.
836	+ 0.66	< 0.05	S.
838	+ 0.33	> 0.05	N.S.

S. = Significant.
H.S. = Highly Significant.
N.S. = Not Significant.

TABLE 42.

COMPARISON OF THE DAILY MEANS OF ANTIBODY TITRES BETWEEN GROUPS 1 AND 2.

Days Post-Challenge	Degree of Freedom	t	P	Inter-pretation
0	19	1.04	> 0.400	N.S.
1	19	1.04	> 0.400	N.S.
2	19	0.19	> 0.900	N.S.
3	15	1.27	> 0.300	N.S.
4	11	0.38	> 0.800	N.S.
5	15	3.01	< 0.010	S.
6	15	1.15	> 0.300	N.S.
7	15	4.11	< 0.001	H.S.
8	15	3.75	< 0.010	H.S.
9	15	2.96	< 0.010	H.S.
10	19	2.77	< 0.020	H.S.
11	19	3.94	< 0.001	H.S.
12	15	4.88	< 0.001	H.S.
13	19	5.28	< 0.001	H.S.
14	19	5.66	< 0.001	H.S.
21	19	5.29	< 0.001	H.S.
28	19	1.27	< 0.001	H.S.
35	19	4.04	< 0.001	H.S.

S. = Significant.

N.S. = Not Significant.

H.S. = Highly Significant.

TABLE 43.

GAMMAGLOBULIN Vs ANTIBODY TITRES GROUP 3.

Number of Animal	N	r	P	Inter-pretation
39	16	+ 0.283	> 0.05	N.S.
40	16	+ 0.095	> 0.05	N.S.
52	15	+ 0.671	< 0.01	S.
65	15	+ 0.692	< 0.01	S.
67	15	- 0.42	> 0.05	N.S.
63	15	0.30	> 0.05	N.S.
68	15	- 0.10	> 0.05	N.S.
Days Post-Challenge				
0	7	- 0.41	> 0.05	N.S.
7	7	- 0.268	> 0.05	N.S.
14	7	- 0.35	> 0.05	N.S.
21	7	- 0.59	> 0.05	N.S.
28	7	- 0.23	> 0.05	N.S.
35	7	- 0.12	> 0.05	N.S.
Mean Values	16	0.67	< 0.01	S.

S. = Significant.

N.S. = Not Significant.

TABLE 44

IgG_1 Vs ANTIBODY TITRES

No. of Sheep	N	r	P	Inter-pretation
39	16	0.34	> 0.05	N.S.
40	16	0.64	> 0.05	N.S.
52	15	0.14	> 0.05	N.S.
65	15	0.64	< 0.01	S.
67	15	0.15	> 0.05	N.S.
63	15	-0.16	> 0.05	N.S.

Days Post-Challenge	N	r	P	Inter-pretation
0	7	-0.66	> 0.05	N.S.
7	7	-0.41	> 0.05	N.S.
14	7	-0.55	> 0.05	N.S.
21	7	-0.79	< 0.05	S.
28	7	-0.83	< 0.05	S.
35	7	+0.11	> 0.05	N.S.
Mean Values	10	+0.67	< 0.01	S.

S. = Significant.

N.S. = Not Significant.

TABLE 45.

MEAN DIFFERENCE OF ANTIBODY TITRES (DAY 0 - DAY X)

OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	p	Inter-pretation
1	0.00	0.00	0.00	> 0.900	N.S.
3	0.00	0.00	0.00	> 0.900	N.S.
5	0.00	0.00	0.00	> 0.900	N.S.
6	- 0.50	0.50	1.00	> 0.400	N.S.
7	- 0.28	0.29	1.00	> 0.400	N.S.
8	- 0.40	0.40	1.00	> 0.400	N.S.
9	- 0.71	0.47	1.52	> 0.200	N.S.
10	- 0.86	0.46	1.86	> 0.200	N.S.
11	- 1.71	0.28	6.12	< 0.001	H.S.
12	- 1.86	0.34	5.46	< 0.001	H.S.
13	- 1.57	0.40	3.90	< 0.010	H.S.
14	- 1.86	0.24	7.75	< 0.001	H.S.
21	- 2.00	0.22	9.09	< 0.001	H.S.
28	- 2.00	0.41	4.84	< 0.001	H.S.
35	- 2.00	0.41	4.87	< 0.001	H.S.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 46.

MEAN DIFFERENCE OF ANTIBODY TITRES (DAY 0 - DAY X) OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	0.00	0.00	0.00	> 0.90	N.S.
2	- 0.60	0.40	1.50	> 0.20	N.S.
3	- 0.40	0.40	1.00	> 0.40	N.S.
4	- 0.40	0.40	1.00	> 0.40	N.S.
5	- 0.40	0.40	1.00	> 0.40	N.S.
6	- 0.40	0.40	1.00	> 0.40	N.S.
7	- 0.40	0.40	1.00	> 0.40	N.S.
8	- 0.40	0.40	1.00	> 0.40	N.S.
9	- 0.40	0.40	1.00	> 0.40	N.S.
10	- 0.60	0.60	1.00	> 0.40	N.S.
11	- 0.60	0.40	1.50	> 0.20	N.S.
12	- 0.40	0.40	1.00	> 0.40	N.S.
13	- 0.40	0.40	1.00	> 0.40	N.S.
14	- 0.40	0.40	1.00	> 0.40	N.S.
21	- 0.40	0.40	1.00	> 0.40	N.S.
28	- 0.40	0.40	1.00	> 0.40	N.S.
35	- 0.40	0.40	1.00	> 0.40	N.S.

N.S. = Not Significant.

DISCUSSION

Serum Protein Patterns in Sheep before Infection and Re-infection with Orf Virus

The levels of total serum proteins, albumin and globulin fractions reported in the literature range widely; my values fell within the extremes where comparisons were meaningful (Table 47).

The total serum protein values determined in the present work were higher than those recorded by Kuttler and Marble (1960), Dobson 1966), Halliday (1966), Khalaf (1978), and Shubber (1978), similar to those reported by Becker and Smith (1950), Egan and Cuill (1971) and Singh and Dutt (1974), and lower than those recorded by Weaver (1974). The quantitative variations reported have been attributed to variations in technique and specific factors such as age, breed, nutritional state and health of the animals (Becker and Smith 1950; Ravaioli 1959; Perk and Lobl 1960). Perk and Lobl (1960), for example, found that the total serum proteins increased with age and that this increase was primarily in the globulin fractions which they attributed to antigenic stimulation from the diet, and from pathogenic and non-pathogenic infections. In my studies, a significant drop in the total serum proteins was observed in the unchallenged previously infected sheep during the period of the experiment attributable perhaps to excitement from handling because no corresponding decreases were observed in the albumin and globulin fractions. In contrast, the

TABLE 47.

Normal Serum Protein Values Compared to Recorded Values

Total Serum Proteins (g/l)	Albumin (g/l)	GLOBULINS (g/l)							References
		$Alpha_1$	$Alpha_2$	Beta	Gamma	I_gM	I_gG_1	I_gG_2	
72.70 – 80.50									Becker and Smith (1950)
56.50 – 73.60	32.00 – 38.30	1.30 – 2.30	2.20 – 2.90	3.70 – 5.50	6.20 – 18.80				Perk and Lobl (1960)
58.10 ± 0.54	29.60								Kuttler and Marble (1960)
65.20 ± 0.02	35.70 ± 0.01	2.80 ± 0.01		3.60 ± 0.01	12.90 ± 0.10				Dobson (1966)
60.10 – 71.90					25.10				Halliday (1966)
67.80 ± 7.90	18.90 ± 5.20								Egan and Cuill (1971)
85.00 ± 8.00	30.00 ± 6.00								Weaver (1974)
73.00 ± 0.04	16.50 ± 0.04								Singh and Dutt (1974)
						2.07 ± 0.16	14.00 ± 0.52	3.24 ± 0.20	Ciupercescu (1977)
62.00 – 65.40					17.60 – 20.10	4.00 – 5.00	15.00 – 15.50	6.00 – 7.00	Khalaf (1978)
56.90 – 60.20					17.10 – 19.70	4.50 – 4.57	11.40 – 13.50	4.00 – 4.60	Shubber (1978)
62.2 ± 7.40									Boss, Gerber and Tschudi (1979)
66.90 – 74.00	30.90 – 36.10	3.16 – 3.90	8.30 – 9.30	4.34 – 6.66	18.40 – 21.80	3.05 – 3.60	11.50 – 15.80	4.40 – 5.70	Present Project

total serum protein levels in the challenged sheep remained the same or rose slightly.

The gammaglobulin values detected in the sheep before infection or re-infection were higher than those reported by Dobson (1966) but approximated the values published by Khalaf (1978), Shubber (1978), Halliday (1966) and Perk and Lobl (1960) in mature clinically normal rams and ewes.

In the immunoglobulin classes, the IgM values recorded in the present study were lower than the values reported by most authors (Table 47) but the IgG1 and IgG2 levels were within the ranges recorded by Ciupercescu (1977), Khalaf (1978) and Shubber (1978).

Orf antibodies were detected in only eight out of the 21 previously infected sheep (38 percent), a finding that is in agreement with Romero-Mercado (1969) who reported that in primary orf infections antibodies were low and often transient.

Serum Protein Changes in Sheep after Infection and Re-infection with Orf Virus

The changes I observed in the serum protein patterns in sheep after infection or re-infection with orf virus indicated that antigenic stimulation had induced a humoral immune response.

Total serum protein values did not change significantly in either the infected susceptible sheep or challenged previously infected sheep. Doxey (1971) reported

that in mature animals the total serum protein levels were maintained at a constant level but could be rearranged in some acute infections and in early stages of parasitisms, mainly as a result of changes in the globulin fractions. Increase in globulin fractions after infection was first reported by Earle (1935). Since then, several authors have reported that globulin fractions particularly the gamma-globulins rose in acute infections (Campbell 1957; Vesselinovitch 1959; Perk and Lobl 1960). Vesselinovitch (1959), moreover, noted that in some viral infections the total serum protein levels remained unaltered but the ratio of the various protein fractions changed. Likewise, I found that although the total serum protein contents did not change, the gamma-globulin fraction increased significantly in the sheep challenged with orf virus. In contrast, in infected susceptible sheep insignificant increases were detected. It is thus tempting to suggest that the changes observed in the gamma-globulin fraction of the susceptible and previously infected sheep challenged with orf virus are due to antigenic stimulation by orf virus.

Smith, Dawson, Wells and Burrells (1976) observed that IgM concentration in sera of lambs infected with parainfluenza 3 virus increased rapidly three days after aerosol challenge and the increase coincided with the appearance of serum antibody to the virus. In contrast, I did not detect any significant changes in the IgM levels in the sera of the susceptible sheep infected with orf

virus. The changes could have been qualitative but the weakness of my study was that specific IgM orf antibody activity was not investigated, hence valid conclusions of lack of IgM participation cannot be drawn.

Smith and his colleagues (1976) also detected higher concentrations of IgG in lambs three weeks after vaccination with live parainfluenza virus 3 and a sharp increase in the IgG levels in vaccinated lambs after an aerosol challenge. Likewise, in the current study, significant increases were noted in the IgG1 and IgG2 levels of sheep re-infected with orf virus and less pronounced increases were detected in sheep exposed to orf virus for the first time.

The rate of orf antibody production in infected susceptible sheep was significantly slower than the rate in the challenged previously infected sheep ~~challenged~~ and the antibody titres of the primary response were significantly lower than the titres attained in the secondary response even in the previously infected sheep which had no detectable antibodies at the time of challenge. In short, challenge of previously infected sheep induced a classical anamnestic reaction (Glenny and Sumersen 1921; Uhr and Finkelstein 1967).

Romero-Mercado (1969) found that most lambs naturally or experimentally infected with orf virus developed transient orf antibodies detectable by precipitation tests within two weeks and detectable by CFT within four weeks. Lutu (1971) in contrast, reported that weaned lambs

developed orf antibodies detected by CFT in the first week after challenge and attained peak titres at an average of five weeks after challenge. In the present work, likewise I found that the 'onset' of orf antibodies as detected by passive haemagglutination tests occurred towards the end of the first week after primary infection but, in contrast, the peak value of antibody titres was reached three weeks later.

Romero-Mercado, (1969) and Lutu, (1971) found that re-infection of sheep with orf virus induced an anamnestic antibody rise clearly demonstrable by precipitation tests in the first week after challenge. Furthermore, Lutu (1971) found that in the secondary response higher antibody titres were obtained and peak values were attained three to four weeks after challenge. The current data likewise, showed that in the secondary immune response to orf virus, antibodies appeared within five days after challenge and reached a peak value in the second week. In addition, the present work revealed that higher antibody titres were detected in the anamnestic response.

The highly significant correlation between the gamma-globulin values and orf antibody titres in both the primary and secondary orf immune response in the present study strongly suggest that the increases detected in the gamma-globulin levels were due to orf antibody production and the equally significant correlation between the IgG1 levels and the orf antibody titres suggest that the antibodies were associated with IgG1.

Hence, in summary, infection of susceptible sheep with orf virus induced a slow and weak primary antibody response. Re-infection evoked a swift and powerful secondary antibody response probably associated with the IgG_1 class of immunoglobulin.

CHAPTER FIVE

STUDIES OF THE CELL-MEDIATED IMMUNE RESPONSES

INTRODUCTION

Evidence has been accumulating on the importance of the role played by cell-mediated immunity (CMI) in the defence of the host against several viral infections, especially membrane-associated viruses such as poxviruses, myxoviruses, herpesviruses and adenoviruses (Boulter 1969; Tompkins, Zarling, and Rawls 1970; Blanden 1974). One theory put forward to explain the necessity of CMI was that CMI promoted recovery from infection by eliminating or restricting virus-infected cells whereas antibodies which are incapable of penetrating cells, provided protection against virus infection by prohibiting the establishment and the spread of the extracellular virus (Bloom and Ragar-Zisman 1975).

Since the observation by Rich and Lewis (1932) that the migration of cells from tissue explants of sensitized animals was inhibited by the sensitizing antigen and the discovery by Landsteiner and Chase (1942; 1945) that delayed hypersensitivity could be transferred to normal recipients by sensitized cells from sensitized donors, considerable advances have been made in the techniques for studying cellular reactions in CMI. The effect or mechanisms of CMI are complex and, as yet, are incompletely understood. Lymphocytes and macrophages are the major cell types involved in CMI; lymphocytes possess immunological specificity and efficacy whereas macrophages are the executors of CMI reactions (Elves,

Roath and Israels 1963; Pearmain, Lycette and Fitzgerald 1963; Mackaness 1969; Bloom 1971). It has also been shown that thymus-dependent lymphocytes (T-cells) are required for cell-mediated immune responses (Miller, Marshall and White 1962; Jankovic, Waksman and Arnasson 1962; Good, Dalmasso, Martinez, Archer, Pierce and Papermaster 1963; Paterson 1966; North 1973).

In 1963, Pearmann and his colleagues observed that when a population of sensitized lymphocytes were stimulated by the sensitising antigen in vitro, morphological and biochemical changes occurred in the small lymphocytes which culminated in an increase in their size and their proliferation. This finding was corroborated by Elves and his colleagues (1963). Furthermore, when lymphocytes were stimulated by sensitizing antigen they produced mediators known as lymphokines which were non-antibody multifactorial amplifiers involved in non-specific recruitment and regulation of circulating lymphocytes, macrophages and polymorphonuclear leucocytes (PMN) (Dumonde, Wossencroft, Parayi, Matthew and Morley 1969).

The well-documented lymphokines are blastogenic (mitogenic) factor (Kasakura 1970) which induces mitosis and proliferation of other lymphocytes thus creating a large population of sensitized lymphocytes, macrophage migration inhibition factor (MIF) (Bloom and Bennet 1966; David 1966), leucocyte migration inhibition factor (LIF) (Soborg and Bendixen 1967; Rocklin 1974; Benditzen

1977) lymphotoxins which induce cytotoxicity of target cells (Ruddle and Waksman 1968), transfer factor which converts non-sensitized lymphocytes to the antigen responsive state (Lawrence 1969; 1971), and chemotactic factors which lure macrophages and PMN leucocytes to the site of infection (Nathan et al., 1971; Repo et al., 1978). Rocklin (1976) tried to explain the functions of the lymphokines by suggesting that chemotactic factors were synthesized to recruit the macrophages and microphages to the reaction site and, once present, these cells were then confined to the site by the migration inhibition factors. Macrophages were then activated to an enhanced phagocytic state by activating factor, indistinguishable from MIF and other lymphocytes were recruited to participate in the reaction by mitogenic factor and transfer factor.

The relevance of in vitro models of CMI mechanisms to in vivo events have been studied and parallelism has been well-documented. The most common in vitro systems used to demonstrate CMI are migration inhibition tests and lymphocyte blast transformation tests. In addition, the successful passive transfer of sensitized lymphocytes or transfer factor to a susceptible recipient with non-sensitized lymphocyte population, is a useful in vivo indicator of CMI.

Migration Inhibition

Migration inhibition tests measure the effects on

target cell population (macrophages or PMN leucocytes) of factors elaborated by sensitized lymphocytes when they are stimulated by the sensitizing antigen. The basic concept of inhibition of cell migration was first pioneered by Rich and Lewis (1928) who had observed that when cells in a plasma clot from tuberculous guinea pigs were cultured in a medium containing tuberculin they were prevented from migrating, whereas, when cells from normal guinea pigs were cultured in the tuberculin-containing medium ~~they~~ were not prevented from migrating. George and Vaughan (1962) developed a capillary tube method for quantitating the migration of macrophages. Their technique was based on observing the migration of sensitized peritoneal exudate (PE) cells, which comprised approximately 70 percent macrophages and 20 percent lymphocytes, from capillary tubes which were placed in chambers containing medium. The tendency of the cells to migrate was curtailed when the sensitizing antigen was included in the medium. David, Al-askari, Lawrence and Thomas (1964) quantified the migration inhibition test using PE cells for assaying the macrophage migration inhibition factor (MIF) and thus produced a meaningful bench test which has been used to demonstrate CMI *in vitro* with numerous antigens e.g. PPD, histoplasmin, coccidion ((Thor *et al.*, 1968), lymphocytic choriomeningitis virus and mumps virus (Tubergen and Oldstone 1971) and vaccinia virus (Tompkins *et al.*, 1970; Hutt 1975)).

In 1967, Soborg and Bendixen described an *in vitro* test based on the inhibition of migration of human peripheral leucocytes from capillary tubes. They observed that when leucocytes from brucella-positive individuals were cultured with brucella antigen their migration was inhibited but the migration of leucocytes from brucella-negative individuals was not inhibited. Later, Rocklin (1974) managed to isolate and characterize the leucocyte migration inhibition factor and found that it was chemically different from MIF. Since then, the leucocyte migration inhibition test has been successfully applied to detect CMI to a variety of antigens. Moreno-Lopez (1977), for example, used the test to show the involvement of CMI in parainfluenza-3 infection in cattle and Hussain and Mohanty (1979) were able to detect a CMI response to bovine rhinovirus type 1 in calves.

Lymphocyte Blast Transformation

Carstairs (1961) first showed that small lymphocytes survived and divided in cultures of human leucocytes. The term 'blast-like' was coined by MacKinney, Stohlman and Brecher (1962) to describe the appearance of the transformed lymphocytes in cultures since they resembled lymphoblasts. The process of this transformation was termed blastogenesis because the transformed cells were capable of mitosis and proliferation (Robbins 1964).

In 1960, Nowell, earlier had observed that the addition of the mitogen, phytohaemagglutinin (PHA), an extract from the red bean, Phaseolus vulgaris, to cultures of normal human leucocytes caused transformation of the lymphocytes into blast-like cells. Similar responses to other mitogens have been observed with normal lymphocytes of other species including sheep (Burells and Wells 1977), cattle (Muscoplat et al., 1974), pigs (Viza et al., 1970) and laboratory animals such as rats, mice, rabbits and hamsters (Knight et al., 1965).

The PHA phenomenon has proved invaluable to immunologists who found that certain antigens also induced a similar transformation in lymphocytes from sensitized individuals. Pearmain and his colleagues (1963) reported that in the presence of PPD, cultures of lymphocytes taken from tuberculin-positive individuals produced mitotic figures similar to those induced by PHA in normal lymphocytes, whereas, cultures of lymphocytes from tuberculin-negative individuals did not show mitosis. Specific antigen-stimulated blast-cells have been observed microscopically with lymphocytes from sensitized individuals and antigens from smallpox vaccine, poliovirus, mumps virus, vaccinia virus, and herpes virus (Elves et al., 1963; Smith et al., 1972; Rosenberg et al., 1972; Hutt 1975).

Lymphocyte transformation has also been shown to be induced by a blastogenic factor which is elaborated by

sensitized lymphocytes on stimulation by the sensitizing antigen (Kasakura and Loweinstein 1965; Gordon and MacLean 1965; Kasakura 1970).

At present, the lymphocyte transformation test measures the blastogenic response of lymphocytes to specific secondary stimulation (i.e. antigens), non-specific stimulation (i.e. mitogens) and the primary response to certain antigens on the cell surface (i.e. allogeneic histocompatibility antigens as shown in the mixed lymphocyte reactions). Although the blastogenic response can be observed microscopically and quantified by counting the proportion of enlarged blast-like lymphocytes with polar staining nuclei and increased basophillic cytoplasm, it is more convenient and precise to determine the extent of blastogenesis by measuring the amount of a DNA precursor taken up by these lymphocytes for DNA replication. This is done by adding radioactive thymidine to the lymphocyte cultures and measuring the amount incorporated into the DNA by scintillation counter. Lymphocytes are separated from other blood or tissue constituents by density gradient technique developed by Boyum (1968) using polysucrose-metrizoate mixture. In this technique the polysucrose agglutinates thus increasing the sedimentation rate of the red cells whilst the metrizoate salt provides the high density required for separating the leucocytes.

Passive Transfer of CMI with Sensitized Lymphocytes

Delayed hypersensitivity reactions are characterized by their inability to be transferred to normal animals by serum from sensitized donors, regardless of the antibody content of the serum. Zinsser and Mueller (1925) first noted this characteristic of delayed hypersensitivity when studying allergies caused by bacteria and used it as a criterion for separating allergies mediated by cells such as tuberculin sensitivity from those mediated by antibodies such as serum sickness.

Frequent uncritical attempts to confer passive sensitivity upon normal animals by transferring cellular elements obtained from sensitized donors of the same species have been made. Landsteiner and Chase (1942) found that the transfer of suspensions of PE cells from guinea pigs sensitized by picryl chloride, a chemical allergen, conveyed a marked delayed hypersensitivity to normal guinea pigs. Lawrence (1949) showed that delayed hypersensitivity to tuberculin antigens and streptococcal proteins could be transmitted to normal human being recipients by intradermal injection of viable peripheral blood leucocytes from sensitive donors. Mackaness (1969) also showed the immune response to infection with Listeria monocytogenes, in mice gave rise to a population of immunologically committed lymphoid cells which had the capacity to confer protection and a proportionate level of

delayed hypersensitivity when transferred to normal mice. Similarly, Blanden (1971) showed that spleen cells from donor mice immunized intravenously with avirulent ectromelia virus, conferred a specific cell-mediated immunity to susceptible mice.

MATERIALS AND METHODS

Experimental Designs

Attempts to detect the involvement of CMI responses in orf infections were made using indirect macrophage migration inhibition (MMI) tests, direct leucocyte migration inhibition (LMI) tests, lymphocyte transformation tests and passive transfer of sensitized lymphocytes to susceptible animals.

The indirect migration inhibition tests were carried out using normal PE cells from guinea pigs to assay the migration inhibition factor (MIF) and leucocytes from normal sheep to assay LIF in cell-free supernate obtained from lymphoid cell cultures of sheep infected with orf virus. Two sheep were re-infected with orf virus by scarifying the right thigh and applying suspensions of orf virus. After nine days the sheep were killed humanely. Lymphocytes were harvested from the prefemoral and popliteal lymph nodes, the spleen and the thymus. PE cells rich in macrophages were produced in two guinea pigs.

The direct leucocyte migration inhibition test was used for assessing the onset and duration of CMI in orf-infected sheep. The following groups of sheep were used:-

Group 3 consisted of eight susceptible sheep challenged with orf virus by scarification; these sheep were also used to study primary humoral responses (see Chapter 4).

Group 4 consisted of 14 previously infected sheep challenged with orf virus by scarification.

Group 5 consisted of ten previously infected sheep which were not challenged.

Blood samples for leucocytes were collected twice a week in heparinized vacutainers.

The lymphocyte transformation test was carried out on lymphocytes from the following two groups of sheep:

Group 6 was made up of six previously infected sheep challenged with orf virus.

Group 7 was also made up of six previously infected sheep which were not challenged.

On days 0, 5, 10, 15, 20, 42, 63, post-challenge blood was collected and lymphocytes for the test separated.

The passive transfer experiment involved the following four groups of sheep:-

Group 8 consisted of four susceptible lambs which were injected with lymphoid cells from sheep re-infected with orf virus nine days previously. Twentyfour hours after this treatment the lambs were injected with 1 ml of a 20 percent orf scab suspension then immediately scarified on the inner side of the right thigh.

Group 9 consisted of three susceptible lambs injected with lymphoid cells from sheep which had not been infected with orf virus. These lambs were similarly infected with orf virus.

Group 10 consisted of three susceptible lambs not given any cells and were infected with orf virus in the

same way as group 8.

Group 11 consisted of four previously infected sheep which were scarified on the inner side of right thigh and orf virus suspension applied.

Lesion developments in the four groups of sheep were compared.

In vitro Tests for CMI

Indirect migration inhibition tests: Two previously infected sheep were killed on the nineth day after challenge and their prefemoral and popliteal lymph nodes, spleens and thymi were collected aseptically into Hank s' balanced salt solution with heparin (HBSS-H) (50 units/ml). Lymphocytes from the organs were harvested by squeezing cut and teased pieces of the nodes, spleens and the thymi separately through a sieve with a mesh number of 200. The cells were washed four times in HBSS-H and their viability and total count determined by the dye exclusion method using 0.3 percent Nigrosin dye. A suspension of 3.5 million cells/ml was made in growth medium consisting of RPMI 1640[1] in hepes buffer with 10 percent heat-inactivated foetal calf serum and containing 100 units of penicillin and 100 mg of streptomycin. The cells were distributed into tissue culture bottles and cell-adapted orf virus was added to some of the bottles; the rest were left as controls. The bottles were then incubated at 37°C for 24 hours after which the cell-free supernatant fluid was harvested and concentrated to 50

1 Flow Laboratories Ltd.

percent of the original volume by dialysis against 40 percent polyglycol ethylene. The concentrated supernate was dialyzed against RPMI 1640 medium, then replenished with 10 percent foetal calf serum and antibiotics before storage at -20°C.

The production of a large number of PE cells was induced in two guinea pigs by injecting them intraperitoneally with a mixture of RPMI 1640 medium and incomplete Freund's adjuvant. Four days later the guinea pigs were anaesthetized with ether and exsanguinated. The animals were then pinned out, their skins reflected and 20 ml of warm HBSS-H introduced into the peritoneal cavity. After gently kneading the abdomen, the cell-rich HBSS-H was collected with a 20 ml syringe. The cells were sedimented out by gentle centrifugation 150 g for 15 minutes, then washed three times in HBSS-H and once in RPMI 1640 medium. After checking viability, a suspension of 10^7 cells/ml was prepared in the RPMI 1640 growth medium and drawn into capillary tubes. One end of the tube was sealed with Cristaseal[1] and then the tubes were centrifuged at 2,000g[2] for five seconds. The tubes were cut precisely at the cell-fluid interface using a diamond glass marker and then one tube containing the cells was placed in each chamber of a migration plate[3]. The end of the tube was fixed onto the chamber wall with a blob of silicone grease and the tube itself was similarly

1 German Hawskley Ltd. 2. Microhaematocrit Centrifuge.

3 Sterilin.

fixed onto the floor of the chamber with a second blob of grease, then the concentrated cell-free supernatant fluid from storage warmed to 37°C in a waterbath was added to each chamber. Control chambers contained the supernatant fluid from the lymphocyte cultures into which orf virus was not added. The rims of the chambers were greased and then the chambers were sealed with square coverslips measuring 13 x 13cm and incubated at 37°C in an atmosphere of 5 percent carbon dioxide for 24 hours. Similarly, leucocytes harvested from ten ml of heparinized blood from a normal sheep which had no history of having suffered from orf infection were washed and processed in the same way as the PE cells to assay the presence of LIF in the cell-free supernatant fluid. The image of the tube and the area of the migrating cells was viewed by a photoenlarger[1] and thereby projected onto graph paper. The outline of the cell migration pattern was traced out and the area of cell migration determined by counting the mm squares within the outline. The results were expressed by the migration index (MI) obtained from the equation:-

$$MI = \frac{\text{Area of migration of cells with lymphokine}}{\text{Area of migration of cells without lymphokine}} \times 100$$

Direct leucocyte migration inhibition test: In this test 14 previously infected sheep were challenged with orf virus by scarification and blood for leucocyte separation was collected into heparinized vacutainers by jugular venepuncture. Erythrocytes were removed by inducing

1 De Vere 54 Varion.

osmotic shock with sterile distilled water and restoring the isotonicity within 30 seconds by the addition of 3.5 percent sodium chloride solution. The leucocytes were then washed three times in HBSS-H and the number of the viable cells counted by the dye exclusion method using Nigrosin dye. The washed cells were then suspended in RPMI 1640 growth medium at a concentration of approximately 10^7 cells/ml. The cell suspension was drawn into capillary tubes, processed and fixed into migration chambers. In the chambers 0.1 ml of 10^{-2} cell-adapted orf virus was added and in the control chambers only growth medium was added. The area of migration of the PMN cells was determined in the same way as for the indirect test and the results were expressed as follows:-

$$MI = \frac{\text{Area of migration of leucocyte with antigen}}{\text{Area of migration of leucocyte without antigen}} \times 100$$

Lymphocyte transformation test: Ten ml of blood was collected from sheep by jugular venepuncture into vacutainers containing 50 units of preservative-free heparin. The blood was thoroughly mixed with the heparin then centrifuged at 1610 g for 30 minutes. The buffy coat formed was harvested into siliconized test-tubes and diluted with an equal volume of 0.15N sodium chloride solution before being layered gently onto lymphocyte isolating fluid made up of 9.6 percent sodium metrioate and 5.4 percent Ficoll (lymphoprep)[1] in conical centrifuge tubes in the proportion of four parts of buffy coat suspension to three parts of

1. Nyegaard & Co., Oslo.

the "lymphoprep". The tubes were spun at 400 g for 30 to 40 minutes. The lymphocytes, which formed a white band at the interface of the saline and "lymphoprep" were carefully pipetted into siliconized glass universal bottles then washed three times in HBSS-H. The viable cells were estimated using the Nigrosin dye exclusion method and then suspended in growth medium composed of RPMI 1640 in hepes buffer, 10 percent heat inactivated horse serum[1], 200 mmol of glutamine, 100 units/ml of penicillin and 100 mg/ml of streptomycin. The final concentration of the cells was 10^6 cells/ml.

The lymphocytes suspension was distributed into wells of a flat-bottomed tissue culture microplate[2] using a volume of 0.2 ml per well. The stimulant (PHA or antigen) was added as required and then the plates incubated at 37°C in an atmosphere of 5 percent carbon dioxide for the desired period, after which the cultures were pulsed with titriated thymidine[3] of 5 Ci/mmol specific activity at 2 µCi per well. The culture growth was terminated 18 to 24 hours later by the addition of cold phosphate buffered saline (PBS) at pH 7.3.

The cultured and labelled lymphocytes were washed twice in PBS and then precipitated with 6 percent trichloroacetic acid aqueous solution overnight at 4°C. The precipitate was dissolved in NCS solubilizer, a quaternary ammonium base in toluene[4] and then mixed with 5 ml of scintillator in glass vials. The scintillator was toluene

1. Gibco Biocult Ltd. 2. Nunc Ltd.
3. Radiochemical, Amersham. 4. Ammersham.

with two standard phosphors, the primary phosphor was 2,5-diphenyloxazole (PPO) at 0.5 percent and the secondary phosphor was 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP)[1].

The amount of tritiated thymidine incorporated into the dissolved cell solution samples was determined by using a beta-emmitting scintillation counter[2]. The counter equipped with photomultiplier, picked up the energy released into the scintillator when photons left the radioisotope. The glass vials with the samples were placed in the machine set to count automatically each sample for one minute. A blank control containing the solubilizer and the scintillator was also included.

A preliminary trial was run to check whether orf antigen could stimulate sheep lymphocytes in vitro and to ascertain the optimal conditions for stimulation. A sheep which had recovered from orf was the source of sensitized lymphocytes and a susceptible lamb not yet infected with orf virus was the source of non-sensitized lymphocytes. Lymphocytes were separated from blood samples of the two animals by the use of the "lymphoprep" method and their cultures set up in 96-well microplates. Orf virus passaged eight times in lamb testis cells and inactivated by 10 percent formalin was serially diluted in PBS using the ten-fold steps and 20 µl of each dilution was added into two wells of cell cultures. PHA was put into twelve other wells of cell cultures as positive controls and growth medium only added to the non-stimulated

1. BDH Chemicals. 2. Tracelab ICN.

cultures. The cultures were incubated for varying periods over seven days, and 18-24 hours before the end of each period tritiated thymidine was added. Culture growth was then terminated by the addition of cold PBS, processed and counted. Maximal transformation of 10^6 lymphocytes/ml occurred when the orf antigen diluted to 10^{-2} was incubated for five days (Figure 9 and Table 51).

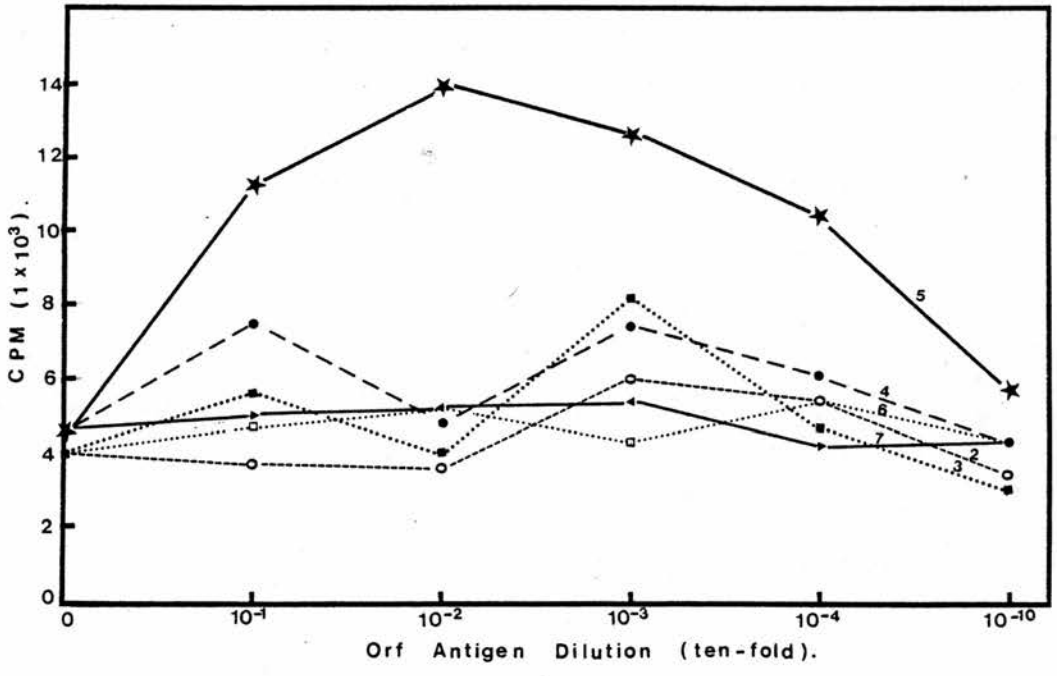
In the test proper two groups of previously infected sheep consisting of six animals each were used. Preliminary investigations of the optimal conditions for the lymphocyte culture system were determined using different concentrations of PHA, whole blood and pure lymphocyte cultures from the two groups of sheep. Peripheral lymphocytes were separated from blood samples and cultured in 96-well tissue culture microplates. A freeze-dried purified PHA[1] reconstituted in distilled water to contain 0.4 ug/ul was added to the cultures as follows:-

0.10 ul/ml, 0.25 ul/ml, 0.50 ul/ml, 0.75 ul/ml, 1.0 ul/ml and 2.5 ul/ml. The cultures were set up in duplicate. Two wells of cultures received only growth medium and acted as non-stimulated cell controls. After three days incubation period, the cultures were pulsed with tritiated thymidine and harvested on the fourth day. They were then processed and counted. The results obtained as counts per minute (cpm) were calibrated on a graph paper and the optimum concentration required to stimulate lymphocytes from the sheep was worked out. Whole blood

1. Wellcome Laboratories Ltd.

Figure 9: Counts per minute of H^3Tdr uptake by lymphocytes cultured with varying concentrations of orf antigen:-

2 - Two days incubation period.
3 - Three days incubation period.
4 - Four days " "
5 - Five days " "
6 - Six days " "
7 - Seven days " "

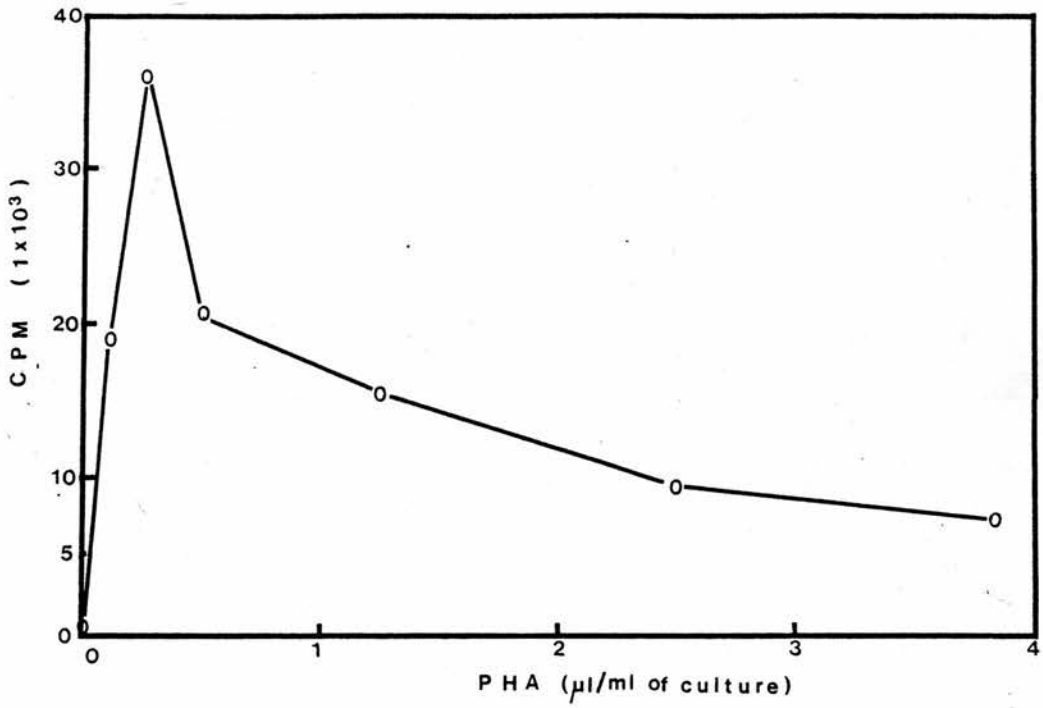


cultures were prepared as described by Bolbol (1975), by diluting blood samples with RPMI 1640 growth medium to contain 10^6 lymphocytes/ml then distributed into microplate at 0.2 ml per well. On harvesting the lymphocytes after pulsing with tritiated thymidine, the red cells were lysed by cold 3 percent acetic acid and then the labelled lymphocytes were washed and processed for counting.

The optimal conditions for the PHA-dose response of the lymphocytes obtained from the two groups of sheep were found to be 0.25 ul/ml of culture for a concentration of 10^6 lymphocytes/ml using pure lymphocyte cultures. The count at this concentration was found to be 35,933 $\pm$ 12035 (Figure 10 and Table 58).

The actual test to determine the onset of CMI in the sheep re-infected with orf virus by assaying the responsiveness of their lymphocytes to orf antigen in vitro was carried out. One group, Group 6, was challenged with orf virus by scarification and the second group, Group 7 which was not challenged acted as the control. Lymphocyte cultures were made from blood samples collected on days 0, 5, 10, 15, 20, 42 and 63 after challenge. Nine cultures were prepared from each sample; three cultures were stimulated by orf antigen ; three stimulated by PHA and three left as non-stimulated cultures. After incubation period of four days, the cultures were pulsed with tritiated thymidine and then their growth terminated

Figure 10 Counts per minute of H^3Tdr uptake by lymphocytes cultured in different concentrations of PHA.



on the fifth day. The cells were then harvested, and processed for counting. Counting done, the results were expressed as a stimulation index (SI) calculated as follows:-

$$SI = \frac{\text{cpm of cultures with orf antigen}}{\text{cpm of cultures not stimulated}}$$

Passive Transfer of CMI by Sensitized Lymphocytes:

Sensitized lymphocytes to orf virus were obtained from the spleen, the thymus and prefemoral and popliteal lymph nodes draining the booster area of a sheep which had been challenged with orf virus by scarification and which was killed on the ninth day when the MI was 45 percent and SI was 3.75. Non-sensitized lymphocytes were obtained from an unchallenged sheep; $^{M1}/_{89}$ percent and SI 1.15. The lymphocytes were harvested as described in the indirect migration inhibition test. A cell suspension of 10^{10} cells/ml was made in RPMI 1640 growth medium. Four susceptible lambs (Group 8) with non-sensitized lymphocyte population (SI below 1) were injected with ten ml of the sensitized cell suspension, five ml intravenously and five ml subcutaneously.

Three susceptible lambs of group 9 were similarly injected but with non-sensitized lymphocytes. Another three susceptible lambs (Group 10) were not injected with any cells. Twentyfour hours after administration of the cells all the lambs were injected intravenously with 1 ml of a 20 percent suspension of an orf scab and

immediately scarified on the inner side of the right thigh. The animals were observed daily for the onset and course of the lesions on the scarified area and other parts of the body.

Four previously infected sheep were also challenged with orf virus by scarification at the same time and the onset and course of the lesions compared to those of the lambs.

RESULTS

Indirect Migration Inhibition Tests

Lymphocytes from the spleens and popliteal and prefemoral lymph nodes from orf infected sheep produced lymphokine, MIF, on stimulation with orf virus *in vitro* because the areas of migration of the PE cells when cultured in the supernates from the orf virus-stimulated lymphocyte cultures from the two sheep ranged from 52 sq mm to 62 sq mm whereas the areas of migration of the PE cells cultured in the supernates from unstimulated lymphocyte cultures from the same two sheep ranged from 111 sq mm to 126 sq mm. In addition, the cells in the supernate from the stimulated lymphocytes were clumped together while those in the supernate from unstimulated lymphocytes were dispersed and spread out (Plate 12).

There was little difference between MIF production by the cells harvested from lymphnodes and those harvested from spleens, the migratory indices being 46 and 50 percent from supernate from lymph node cells and 43 and 54 percent for the supernate from the spleen cells (Table 48).

Inhibition was also observed when normal sheep leucocytes were cultured in the same cell-free supernates from the orf virus-stimulated lymphocytes. The areas of migration of the leucocytes ranged from 45 to 70 sq mm for supernate from orf-stimulated lymphocytes and from 102 to 188 sq mm for the supernate from the unstimulated lymphocytes / (Plate 13). Thus LIF was produced by the lymphocytes from

Plate 12 The migration of PE cells from Guinea pigs cultured in:-

A - Cell-free supernate from lymphocyte cultures stimulated by orf virus.

B - Cell-free supernate from lymphocyte cultures not stimulated.

Plate 13 The migration of leucocytes from a normal sheep cultured in:-

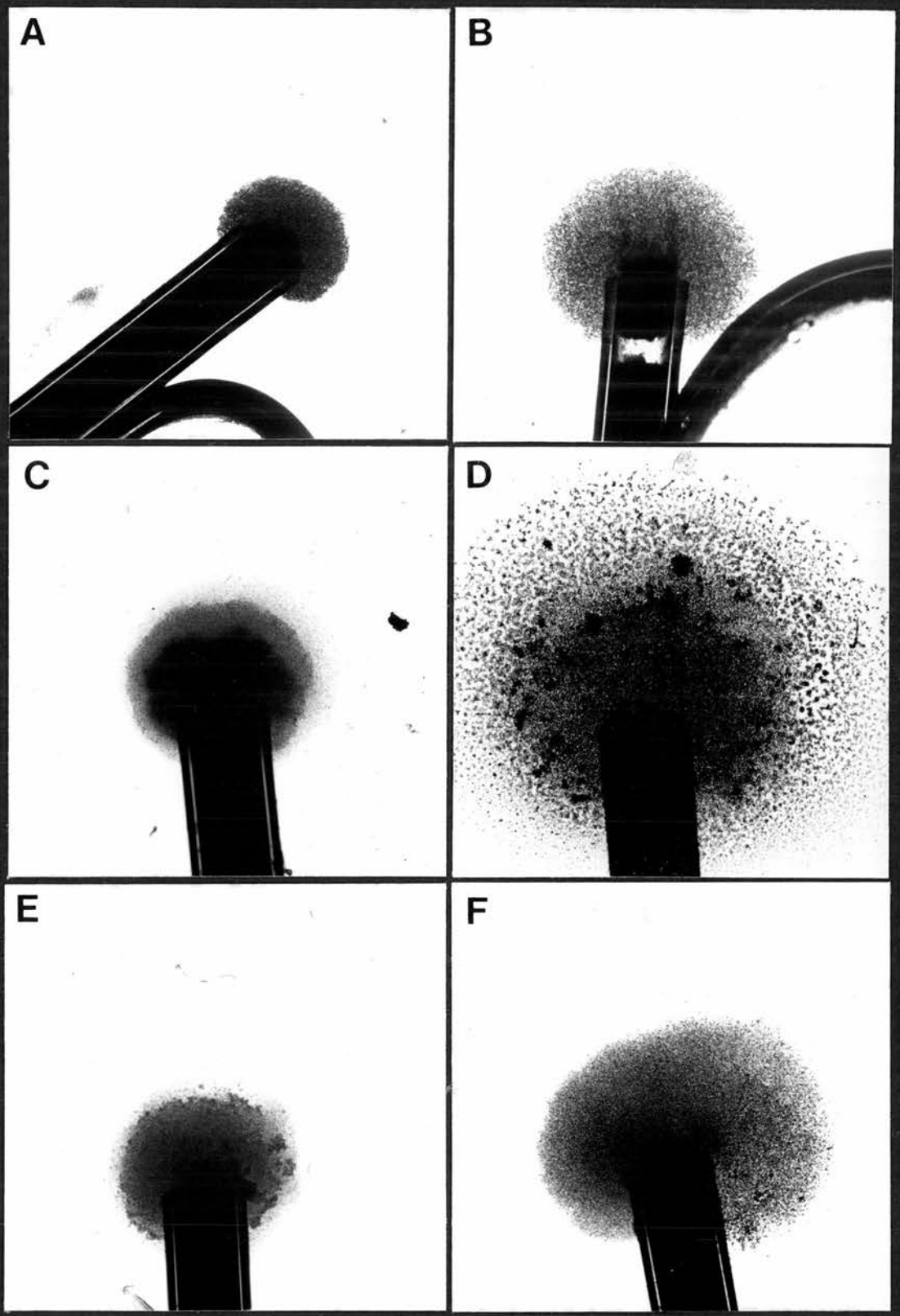
C - Cell-free supernate from lymphocyte cultures stimulated by orf virus.

D - Cell-free supernate from lymphocyte cultures not stimulated.

Plate 14 The migration of leucocytes from sheep re-infected with orf virus when cultured:-

E - Orf antigen containing medium.

F - Growth medium only.


A
B
C
D
E
F

the orf infected sheep.

There was no difference in the production of LIF by the spleen cells or cells from lymph nodes, the migratory indices being 47 and 40 percent for spleen cells supernate, and 37 and 39 for the lymph node cells supernate (Table 49).

On the basis of these preliminary findings, the direct leucocyte migration inhibition test was adopted to determine the onset and duration of CMI responses.

Direct Leucocyte Migration Inhibition (LMI) Tests

The CMI response as evaluated by the LMI tests was detected in both susceptible sheep infected with orf virus and sheep re-infected with orf virus (Figure 8 Appendix Tables 31-38). One consistent feature noted was the variation of the results from animal to animal, day to day, and even within replicates of the same animal on the same day.

In the 14 previously infected sheep which were challenged with orf virus (group 4) the MI before challenge was 79.0 percent indicating that the animals possessed some sensitized lymphocytes due to previous exposure to the virus. The indices decreased after challenge to reach the minimum value of 45 percent 15 days after challenge, and these values remained ~~below~~ about 50 percent for at least seven weeks (Figure 8).

Likewise, the MI obtained from ten previously infected sheep which were not challenged (Group 5) was 66.4

percent on the first day of observation but unlike the challenged sheep of group 4 the indices fluctuated between 66 and 85 percent during the duration of observation. They were always below 100 percent indicating the presence of some sensitized lymphocytes from previous exposure to the virus (Figure 8).

Comparing the results of the challenged previously infected sheep with those of the unchallenged previously infected sheep showed that there was a significant difference from day 4 through to day 63 (Table 50).

In contrast, the MI determined from eight susceptible sheep (group 3) before they were infected with orf virus was high being 94 percent but it dropped to 45 percent on the sixth day after infection, returned to 80 percent on day 8, and declined once more to 61 percent on day 35 (Figure 8).

Comparison of the migratory indices obtained from the infected susceptible sheep with those of the challenged previously infected sheep revealed that the onset of the CMI was similar in both primary and secondary immune responses.

Lymphocyte Transformation Tests

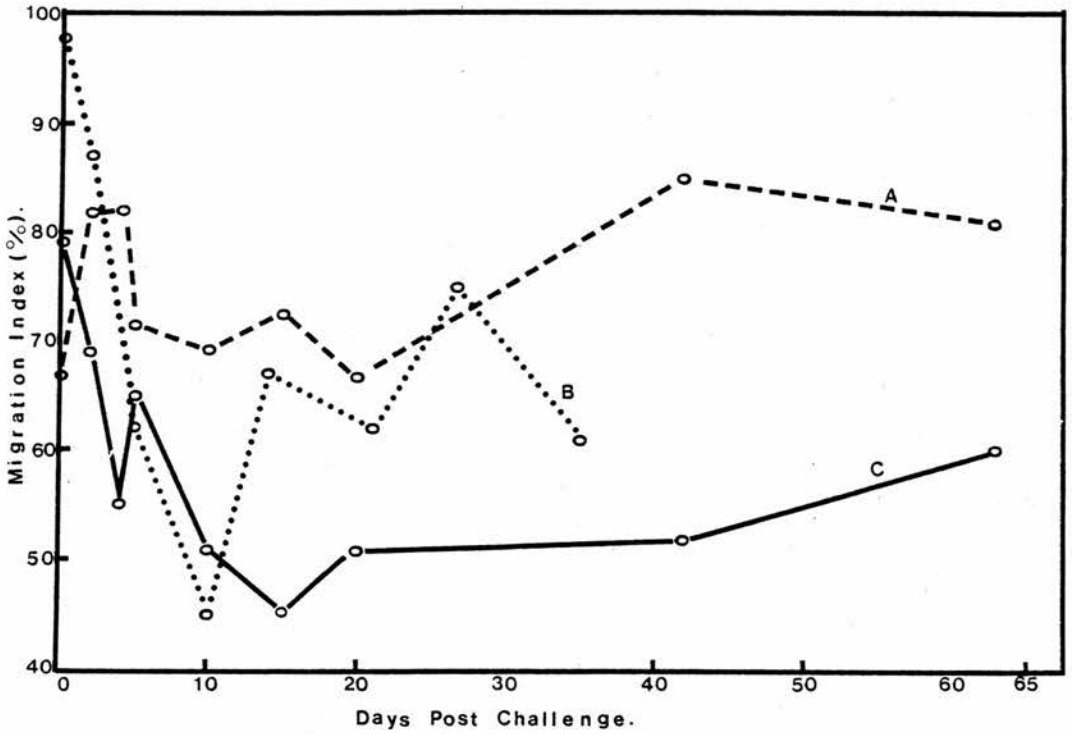
The ability of orf antigen to stimulate lymphocytes in vitro: The uptake of radioactive thymidine by peripheral blood lymphocytes from recently recovered sheep was markedly higher when these lymphocytes were cultured with orf antigen compared to the uptake of the radioactive thymidine by lymphocytes from the same animal

Figure 8 The daily means of the Migration Indices of:-

A - Previously infected sheep unchallenged (Group 5).

B - Susceptible sheep infected with orf virus (Group 3).

C - Previously infected sheep challenged with orf virus (Group 4).



cultured without any stimulant. Thus orf antigen was able to stimulate sensitized lymphocytes in vitro (Table 51). The counts per minute (cpm) for the orf-stimulated lymphocytes was 1,393 and 464 for the unstimulated lymphocytes. There was no increase in the uptake of the radioactive thymidine by the lymphocytes from susceptible lambs; the maximum cpm was 390 for lymphocyte cultured with orf antigen and 443 for unstimulated lymphocyte cultures (Table 52).

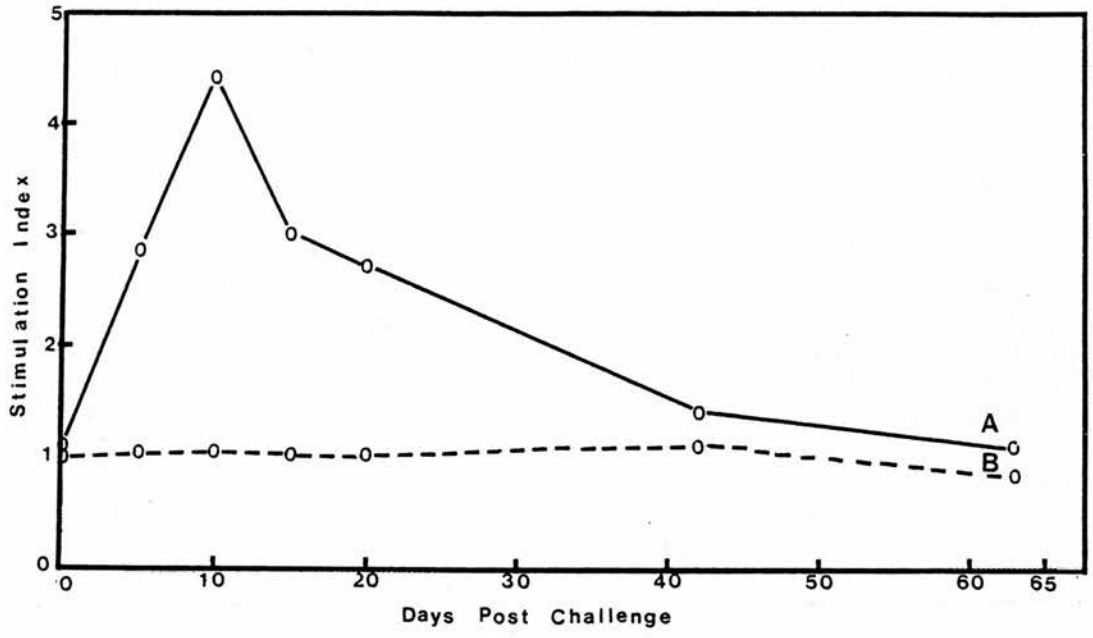
The detection of CMI in sheep re-infected with orf virus: The results of the uptake of radioactive thymidine by orf-stimulated lymphocytes from previously infected sheep in groups 6 and 7 were expressed by SI values which were 1.06 $\pm$ 0.11 for group 6 and 0.96 $\pm$ 0.23 for group 7 before challenge. The SI values increased in the re-infected sheep to reach maximum of 4.34 $\pm$ 1.00 on the tenth day after challenge and then declined gradually to pre-challenge values nine weeks later. In contrast, the SI values for the unchallenged previously infected sheep of group 7 were maintained more or less between 0.99 to 1.14 and these values were significantly different from those of the challenged sheep from day 5 to day 42 after challenge (Tables 53-55 and Figure 11).

The blastogenesis detected in the PHA-stimulated lymphocytes was consistently higher than that detected in the orf antigen lymphocytes or unstimulated lymphocytes.

Figure 11 The daily means of the SI values of:-

A - Sheep re-infected with orf virus (Group 6).

B - Previously infected sheep not challenged (Group 7).



Passive Transfer of CMI using Sensitized Lymphocytes

All the lambs and previously infected sheep reacted to challenge with orf virus (Table 56 Plates 15 - 17). The onsets of the papular, vesicular and pustular stages were similar in the treated and untreated lambs and untreated previously infected sheep but the onsets of scab and resolution stages were highly significantly different between the treated and untreated sheep (Table 56).

Further analysis using the Duncan's multiple-range test at one percent level of probability revealed that the experimental groups fell into the following two distinct subsets in regard to the onset of scab stage:-

Groups 11 8, 9, 10

The mean onset for sheep in group 11 was 6 days and mean onsets for sheep in group 8, 9, 10 was 8.3 days. In other words, the onset of the scab formation in lambs injected with the sensitized lymphocytes was similar to the onsets of the scabs in the susceptible lambs treated with non-sensitized lymphocytes and susceptible lambs not treated with any cells.

Application of Duncan's multiple-range test at the one percent level of probability also showed that the experimental groups fell into two distinct subsets as follows in regards to time of resolution:-

Groups 11, 8 9, 10

The mean time of resolution on groups 11 and 8 was 13.8 days and for sheep in groups 9, and 10 was 28.3 days. In

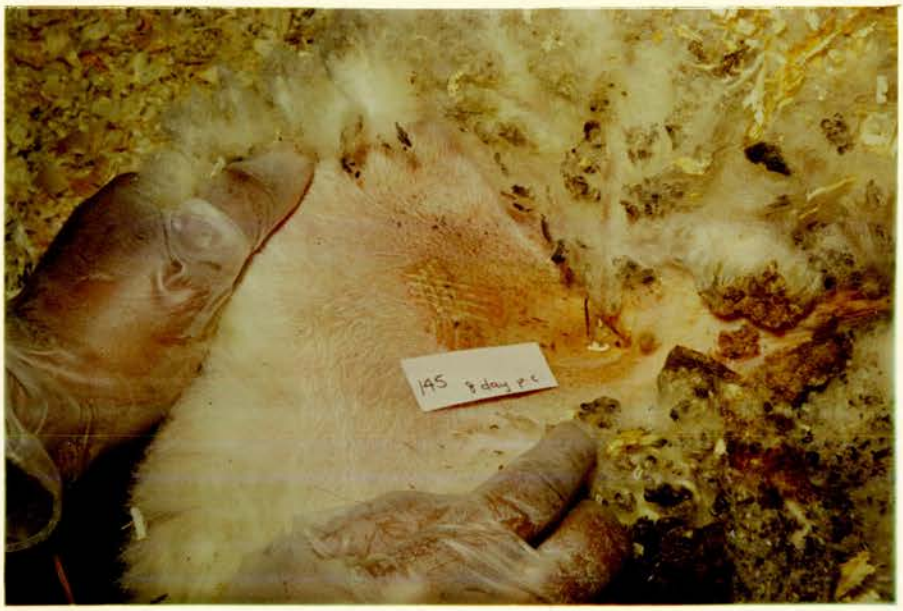
other words susceptible lambs injected with sensitized lymphocytes healed as fast as re-infected sheep.

In addition, three lambs, one from the group of lambs treated with non-sensitized lymphocytes and two from the untreated group of lambs, developed secondary orf lesions on the commissures five days after infection with orf virus.

PLATE 15 Experimental orf lesion on the skin of susceptible lamb treated with sensitized lymphocytes before infection. Eight days after infection.

PLATE 16 Experimental orf lesion on the skin of a susceptible lamb treated with non-sensitized lymphocytes. Eight days after infection.

PLATE 17 Experimental orf lesion on the skin of susceptible lamb eight days after infection.


145 8 days p.c


144 8 days p.c


146 8 days p.c

TABLE 48

AREA OF MIGRATION AND MIGRATORY INDEX OF P.E. CELLS FROM GUINEA PIGS CULTURED IN CELL-FREE SUPERNATES OF LYMPHOCYTE CULTURES.

Source of lymphocytes	NUMBER OF SHEEP					
	691			767		
	Test	Control	MI	Test	Control	MI
Lymph node cells	62	124	50%	58	126	46%
Spleen cells	60	111	54%	52	120	43%

Test - contain supernates from orf stimulated lymphocyte culture.

Control - contain supernate from unstimulated lymphocyte culture.

TABLE 49

AREA OF MIGRATION AND MIGRATORY INDEX OF LEUCOCYTES FROM NORMAL SHEEP CULTURED IN CELL-FREE SUPERNATES OF LYMPHOCYTE CULTURES.

Source of lymphocytes	NUMBER OF SHEEP					
	691			767		
	Test	Control	MI	Test	Control	MI
Lymph node cells	45	115	39%	70	188	37%
Spleen cells	48	102	47%	52	131	40%

Test - contain supernates from orf stimulated lymphocytes.

Control - Contain supernate from unstimulated lymphocytes.

TABLE 50

COMPARISON OF THE DAILY MEANS OF MIGRATORY INDICES BETWEEN CHALLENGED AND UNCHALLENGED PREVIOUSLY INFECTED SHEEP (GROUPS 4 & 5).

Days Post-Challenge	Degrees of Freedom	t	P	Interpretation
0	22	1.51	0.200	N.S.
2	6	1.66	0.200	N.S.
4	6	3.09	0.050	S.
5	12	0.69	0.600	N.S.
10	20	3.21	0.010	H.S.
15	18	5.48	0.001	H.S.
20	12	3.31	0.010	H.S.
42	12	7.15	0.001	H.S.
63	14	2.89	0.020	S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 51.

THE PARAMETER ON ORF-ANTIGEN-DOSE-TIME RESPONSE OF LYMPHOCYTE CULTURES FROM INFECTED SHEEP.

Days post-inoculation (when cells were harvested)	COUNTS PER MINUTE						
	ORF ANTIGEN DILUTIONS					CONTROL	PHA 0.5ul/ml.
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-10}		
2	372.3±43	360± 23	606±243	535.8± 6	357.3± 12	407± 159	7,011.2±4422
3	556 ± 88	398.5± 290	818.75± 219	471.25±34	295.3± 60	397.67 ± 86	8,662.67± 4505
4	750.5 ± 63	481.5± 34	739.5 ± 438	609.5 ±65	313.75±20	451± 428	13,993.67± 2061
5	1130 ± 98	1393 ± 170	1263 ± 89	1037± 141	566.75±90	464± 140	6,858.3± 847
6	472.67 ± 155	522.2 ± 156	429 ± 130	538.8±175	425.5 ±56	390± 68	2,439.8± 341
7	496 ± 301	523.3 ± 197	538 ± 286	423.3±131	521.5±326	461±125	875.5 ± 283

TABLE 52

UPTAKE OF H^3 Tdr BY NON-SENSITIVE LYMPHOCYTES FROM A SUSCEPTIBLE GROUP.

Number of Sheep	PHA- MITOGEN	ORF-ANTIGEN	NO ANTIGEN	SI= $\frac{\text{Antigen}}{\text{Control}}$
143	1,405.5 ± 630	145.25 ± 27	187 ± 3	0.78
144	2,414 ± 112	390.5 ± 26	443 ± 34.2	0.88
145	2,446.5 ± 118	291 ± 17.8	236 ± 38	1.23
147	2,615.5 ± 230.6	189 ± 127	190 ± 0	0.99
148	2,813 ± 193.5	356 ± 54	414.5 ± 17.5	0.86

TABLE 53.

Uptake of H^3 Tdr by Lymphocytes from Sheep Re-infected with Orf Virus – Group 6

Days Post Challenge	COUNTS PER MINUTE			S.I.
	PHA–Mitogon	Orf-antigen	No-antigen	
0	39229 ± 9632	2952 ± 1348	2984.7 ± 1308	1.06
5	40567 ± 23439	4244.5 ± 924	1597.3 ± 596	2.66
10	44015 ± 20555	3209 ± 1416	740 ± 322	4.34
15	40515.5 ± 30002	1998 ± 790	1018 ± 424	1.96
20	41465 ± 32983	29906 ± 1047	1151.3 ± 341	2.60
42	26990.3 ± 18770	1722.5 ± 506	1267 ± 492	1.36
63	62309 ± 44290	1627.7 ± 278	1507.2 ± 153	1.08

TABLE 54. Uptake of H^3 Tdr by Lymphocytes from Unchallenged Previously Infected Sheep – Group 7

Days Post Challenge	COUNTS PER MINUTE			S.I.
	PHA–Mitogen	Orf-antigen	No antigen	
0	17099 ± 6469	1619.75 ± 619	1694.75 ± 768	0.96
5	18875 ± 9979	2251.1 ± 914	2111.9 ± 454	1.07
10	13717.75 ± 2178	1418 ± 317	1370 ± 93	1.04
15	21747.5 ± 7367	1862 ± 1092	1930.3 ± 1236	0.96
20	38574 ± 25305	1555 ± 779	1580.3 ± 664	0.98
42	32238 ± 18899	1722 ± 289	1635 ± 319	1.05
63	39024 ± 20261	1639 ± 142	1436 ± 89	1.14

TABLE 55

COMPARISON OF THE SI VALUES BETWEEN THE CHALLENGED AND UNCHALLENGED SHEEP - GROUPS 6 and 7.

Days Post-Challenge	Degree of Freedom	t	P	Inter-pretation
0	10	0.96	> 0.400	N.S.
5	10	5.29	< 0.001	H.S.
10	8	7.91	< 0.001	H.S.
15	10	6.62	< 0.001	H.S.
20	10	4.89	< 0.001	H.S.
42	10	3.74	< 0.010	H.S.
63	10	1.22	> 0.300	N.S.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 56.

THE DAYS ON WHICH THE STAGES OF THE ORF LESION APPEARED IN LAMBS TREATED AND NOT TREATED WITH SENSITIZED LYMPHOCYTES.

STAGE	Lambs treated With Sensitized lymphocytes (Group 8)				Lambs Treated with Unsensitized Lymphocytes (Group 9)			Lambs not treated with any cells (group 10)			Previously Infected sheep not treated with any cells (group 11)			
	119	120	143	145	118	144	148	141	142	146	67	32	51	126
Papule	3	3	4	3	3	2	2	3	3	2	2	2	2	3
Vesicle	4	4	5	4	4	4	4	5	5	4	4	3	4	5
Pustule	7	4	6	6	7	6	6	6	6	5	5	4	5	6
Scab	8	7	8	8	9	9	9	9	9	8	6	5	6	7
Resolution	14	14	15	21	25	28	24	28	35	30	11	10	11	14
Secondary lesions (√)					√			√	√					

TABLE 57.

SIGNIFICANCE OF DIFFERENCES IN THE ONSETS OF THE ORF LESIONS IN SHEEP TREATED AND UNTREATED WITH SENSITIZED LYMPHOCYTES.

Lesion Stage	Degrees of Freedom	Variance Ratio	P	Inter-pretation
Papule	3, 10	2.80	0.05	N.S.
Vesiscle	3, 10	0.91	0.05	N.S.
Pustule	3, 10	0.24	0.05	N.S.
Scab	3, 10	10.80	0.01	H.S.
Resolution	3, 10	24.97	0.01	H.S.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 58.

THE PARAMETER ON PHA-DOSE RESPONSE OF LYMPHOCYTES FROM 12 ADULT SHEEP

PHA-Dilutions µl/ml	COUNTS PER MINUTE	
	Whole Blood Culture	Pure lymphocyte Culture
0	958 ± 389.0	379.3 ± 222.0
0.1 µl	4,380 ± 1123	19,001 ± 0.000
0.25 µl	9,165 ± 0.000	35,933 ± 12035
0.50 µl	7,808 ± 915.0	20,361.5 ± 2804
1.25 µl	9,527 ± 879.0	15,539.5 ± 8626
2.5 µl	13,666 ± 860.0	9,594 ± 4307
3.75 µl	10,543 ± 847.0	7,350 ± 3942

DISCUSSION

CMI responses are those specific immune reactions which can occur in absence of demonstrable antibodies and can be transferred to normal susceptible recipients only by the transfer of sensitized cells. Since the work of Chase (1945) it has been established that the immunological information for CMI responses resides in T-lymphocytes and macrophages are the main cell type executing the responses. The activities and products of the T-cells *in vitro* have provided insight into the CMI mechanisms *in vivo*. The significance and relevance of *in vitro* systems so far developed to *in vivo* mechanism has been firmly established by several workers who have observed a remarkable correlation in the *in vitro* systems positivity and the presence in the cell donor of a cell-mediated immune state and no correlation with the presence or absence of circulating antibodies (Oppenheim 1968; Bendixen and Soborg 1969; Rocklin *et al.*, 1970; Bloom 1971; Blanden 1974).

In the current study, a consistent feature noted was the marked variation in both LMI and lymphocyte transformation assays from day to day, animal to animal, and even from within replicates of the same animal on the same day. The variations might be due to non-specific factors such as minor changes in technique, differences in time of bleeding, time of setting up the cultures or might be due to specific factors such as pathogenic or non-pathogenic infections. For example, an enhancement

of PMN leucocyte migration instead of the expected inhibition was observed in a sheep that had pasteurella pneumonia (personal observation). Bolbol, (1975) observed a similar variation when studying the effect of Fasciola hepatica extracts on rabbits lymphocytes. Likewise, Hutt (1975) obtained inconsistent results when carrying out MIF tests to demonstrate delayed hypersensitivity in mice infected with vaccinia virus. However, despite the variations, conclusions drawn from the results obtained in the studies of CMI responses in orf infections are believed to be valid.

Migration Inhibition

MIF and LIF were both produced by lymphocytes from a recovered sheep when cultured in vitro in the presence of orf virus because both PMN leucocytes were inhibited in their migration. Moreover, only lymphocytes from orf virus-infected sheep produced LIF in the direct tests. Hutt (1975) earlier had found that spleen cells from mice infected with vaccinia virus produced MIF which inhibited the migration of guinea pig PE cells. Similarly, Shimizu and his colleague (1977) showed that peripheral lymphocytes from African swine fever virus-infected pigs elaborated LIF in vitro in the presence of African swine fever antigen.

Following the demonstration of LIF in the pilot experiment, LMI tests were carried out in sheep with primary orf infection. The CMI responses appeared earlier than the humoral immune responses. The MI was high before

challenge indicating absence of sensitized lymphocytes in the sheep. After infection, the indices dropped reaching the lowest value on10th day indicating the appearance of sensitized lymphocytes in circulation. The occurrence of CMI very early in a primary viral infection was also observed by Hussain and Mohanty (1979) who found that lymphocytes from calves infected with bovine rhinovirus type 1 exhibited typical CMI responses, as detected by LMI tests, as early as the third day after infection and before any humoral antibodies could be detected. Shimizu and his colleagues (1977) however, discovered that pigs infected with African swine fever virus developed CMI responses detected by LMI tests 20 days after infection. Their experimental design is open to the criticism that the sampling gap was too big; the first sample after infection was taken on the sixth day and the next on the 20th day.

The duration of CMI response in sheep with the first exposure to orf virus was long; migratory indices were still low in some sheep 90 days after infection, indicating that sensitized lymphocytes remained in the sheep even after complete recovery. Earlier, Hutt (1975) had also found that in mice infected with vaccinia virus CMI responses as detected by migration inhibition tests, were apparent eight days after infection and were demonstrable for a further 130 days after recovery from infection. However, Hussain and Mohanty (1979) found that the CMI responses in calves infected with rhinovirus diminished by

day 28 after challenge; this could be due to the avirulent nature of their virus and the mild type of infection.

Lymphocyte Transformation

The data from lymphocyte transformation tests indicated that lymphocytes from sheep re-infected with orf virus were specifically stimulated by orf antigen *in vitro* resulting in blast transformation thus providing evidence of CMI responses in infected sheep. The amount of radioactive thymidine uptake by the orf antigen-stimulated lymphocytes was always lower than the amount taken up by PHA-stimulated lymphocytes but always higher than the amount taken by the unstimulated lymphocytes. Gold and Peacock (1970) expressed the current belief that 5 to 30 percent of lymphocytes in a culture are transformed by specific antigen because only relatively small numbers of lymphocytes in the donor body become sensitized to the antigen. On the other hand, non-specific stimulation by the mitogen PHA causes 50 to 70 percent of the lymphocytes in the culture to transform because both T-cell and B-cells are being stimulated (Oppenheim and Rosenstreich 1976). My results are in agreement with these hypotheses because stimulation by PHA was always ~~been~~ greater than by orf antigen.

The onset of the CMI responses as revealed by the lymphocyte transformation test occurred before the humoral immune responses in sheep re-infected with orf virus thus confirming the results obtained by the LMI tests. High SI values were recorded in the first week after re-infection

and reached peak values in the second week then declined to pre-challenge values which also equalled those of the unchallenged previously infected sheep by the nin-th week. The present results are similar to those obtained by Moreno-Lopez (1977) who registered highest SI values in calves re-vaccinated with parainfluenza 3 virus in the second week after the second intranasal vaccination.

In contrast to the LMI tests results, the duration of CMI responses as indicated by lymphocyte transformation tests in sheep re-infected with orf virus was found to last up to the 90th day after re-infection. A tentative explanation could be that a small number of sensitized lymphocytes only are required to produce sufficient lymphokine to inhibit the migration of PMN leucocytes but, on the other hand, a relatively large number of sensitized lymphocytes are required for the blast transformation in the presence of the sensitizing antigen.

Passive Transfer of CMI

CMI responses are passively transferred only with sensitized lymphocytes or transfer factor and not with serum (Bloom and Chase 1967; Lawrence 1971; Blanden 1974). I therefore decided to inoculate susceptible lambs with lymphoid cells from a recovering sheep and to challenge these lambs with orf virus 24 hours later in attempt to induce an accelerated reaction observed in re-infected sheep. The onset of scab formation in the lambs inoculated with sensitized lymphocytes was similar to that of lambs which received non-sensitized lymphocytes and the

untreated lambs. Although the onset of scab formation was significantly different from the onset of scab stage in the previously infected sheep challenged at the same time, the time interval involved is narrow whereas the healing interval was wide. The time of resolution in the lambs injected with sensitized lymphocytes was statistically similar to the resolution time of the sheep re-infected with orf virus. Hence, the indication that a degree of immunity was transferred was, in part, confirmed by the observation that passively immunized lambs never showed any evidence of secondary lesions in the mouth commissures and lips whereas such lesions were found in 50 percent of the lambs not passively immunized. My results are similar to those obtained by Blanden (1970) who showed that spleen cells from mice immunized with ectromelia virus, conferred some degree of immunity to susceptible mice speeding up their recovery after ectromelia virus challenge.

In contrast, Osman (1976) failed to transfer immunity passively to susceptible lambs using spleen cells and thymic cells from a recovered lamb. His experimental design can be critised because he used only two lambs. Moreover, the source of sensitized cells may be crucial. I used four lambs and collected sensitized cells from the prefemoral and popliteal lymph nodes as well as from the spleen and thymus of a mature sheep boosted just before slaughter whereas Osman (1976) used cells from the spleen

and the thymus only of a lamb which had recovered from orf infection. Kircheiner and Weisser (1947) for example, found that cells from the spleen conferred less sensitivity than peritoneal exudate cells.

Another factor considered critical was the number of cells transferred; I used a cell concentration of 10×10^{10} per lamb whereas Osman (1976) used 33×10^{7} per lamb. Turk (1975), for instance, found out that the proportion of sensitized donor cells at the test site was related to the proportion of transferred cells in the peripheral blood. The histocompatibility of the donor and recipient cannot be criticized because same kind of animals were used.

CHAPTER SIX

EXPLORATORY

IMMUNOSUPPRESSION

STUDIES

INTRODUCTION

The fact that immune responses could be suppressed and virulence of pathogens enhanced by cytotoxic drugs or hormones has proved useful in defining the mechanisms by which the immune system works in the clearance of pathogens and the recovery of the host. Several workers have examined the effects of immunosuppressive drugs on viral infections and found that members of herpesvirus, poxvirus, arbovirus, myxovirus and picornavirus groups have manifested enhanced virulence in host animals receiving immunosuppressive therapy; Bugbee, Like and Stewart (1960) for example, found that in cortisone-treated rabbits there was a postponement of the skin lesion development to vaccinia virus infection although the virus was found to replicate in several organs and even led to mortality. Similarly, Nathanson and Cole (1971) reported that immunosuppression converted sublethal West Nile virus infections in adult rats to lethal infections that were manifested by an increased number of infected and destroyed cells of target organs and a pathological picture in which inflammatory response was remarkedly reduced. Narita, Inui, Yabuki, Namba and Shimizu (1979) also found that non-suppurative and pustular vulvovaginitis were induced in calves treated with dexamethasone three months after recovery from primary infectious bovine rhinotracheitis virus infection.

Suppression or destruction of lymphoid cells by immunosuppressive drugs infection was also found to alter both humoral and CMI responses to the pathogen (Bach 1975). Claman (1972) and Zurier and Weissman (1973) earlier reported that corticosteroid therapy suppressed antibody production to antigens, markedly so in steroid-sensitive animals such as mice, rats and rabbits and less markedly in steroid-resistant animals like man, ferrets and guinea pigs. So far as I can ascertain information on whether sheep are steroid-sensitive or steroid-resistant is not available.

Zurier and Weissman (1973) also reported that immunosuppression was more easily achieved in primary immune responses compared with responses to subsequent antigenic stimuli and that the early induction period of the antibody response was much more vulnerable to steroid intervention. Wells (1976) noted that majority of the patients receiving a standard dosage of corticosteroid drugs had reduced levels of IgG in their serum.

Claman (1972) summarized from the small data available concerning the effects of corticosteroids on CMI that, CMI manifestations could be suppressed if large doses or prolonged treatment of the steroid were given. In general, the reports comply in that corticosteroids have no effect on the sensitization of lymphocytes by an antigen nor do they have any effect on stimulation of sensitized lymphocytes by the sensitizing antigen to produce lymphokines (Weston, Claman and Krueger 1973; Casey and

McCall 1971; Bach 1975) but the reports on the effects of corticosteroids on PMN leucocytes and macrophages are discordant. Weston and his colleagues (1973) for example, found that cortisol prevented non-sensitized PE cells of guinea pigs from responding to macrophage activation factor and Casey and McCall (1971) found that macrophages migration inhibition was suppressed in vitro if steroid was administered at the time of sensitization of rabbits with BCG. Stevenson (1973) similarly, found that corticosteroid stimulated the migration of leucocytes in vitro.

It was decided to explore the effect of immunosuppression on the clinical and immunological responses in sheep infected and re-infected with orf virus in an attempt to identify the mechanisms involved in the clearance of the virus.

MATERIALS AND METHODS

Experimental Design

A series of experiments was carried out to follow the lesion development and humoral and cell-mediated immune responses in susceptible and previously infected sheep treated with bethamethasone 24 hours before being challenged with orf virus. The animals were allocated to the following four groups:-

Group 12 consisted of eight susceptible sheep which were injected with betamethasone before being infected with orf virus,

Group 3 consisted of eight susceptible sheep untreated with the betamethasone but infected with orf virus,

Group 13 consisted of eight previously infected sheep which were treated with betamethasone before being challenged with orf virus and

Group 14 made up of eight previously infected sheep not treated with betamethasone but challenged with orf virus.

Immunosuppression

Sheep were weighed before isolation in crates. The immunosuppressant used was soluble betamethasone[1] administered intramuscularly at the dose of 1 mg/kg body weight. The synthetic corticosteroid was injected into the animals

1 Betsolan soluble, Glaxo Ltd.

only once 24 hours before scarification and application of the virus. The lesion development was examined daily using the optical light.

Humoral Immune Response Studies

Total serum protein concentrations, densitometric evaluations of serum, immunoglobulin levels and orf antibody titres were determined as described earlier in chapter four.

CMI Response Studies

Leucocyte migration inhibition tests as described previously in chapter five were performed on the leucocytes from the susceptible sheep given the immunosuppressive drug before challenge (group 12) and simultaneously on leucocytes from untreated susceptible sheep infected with orf virus (group 3).

RESULTS

Clinical Response

The treated and the untreated infected sheep had no obvious systemic signs. The stages of the lesion development took longer to appear in the treated susceptible sheep compared to the appearance of the stages in the untreated susceptible sheep. Papules and vesicles were not evident until the eighth day after infection; pustules appeared on the tenth day and scabbing started on the twelfth day. Complete resolution took six weeks in the treated susceptible sheep whereas it took four to five weeks in the untreated susceptible sheep.

Similarly, the lesion development in the treated re-infected sheep took longer compared to that of the untreated re-infected sheep. Resolution was three weeks in the treated while it was two weeks in the untreated.

Humoral Immune Response Studies

Total serum protein concentrations: The total serum protein contents of the eight susceptible sheep which were given betamethasone treatment before being infected with orf virus (group 12) ranged from 67 to 72 g/l in sera taken before the treatment and from 60 to 82 g/l in the sera taken after treatment and infection (Appendix Table 41). The mean values of the daily samples ranged from 66.10 $\pm$ 2.90 to 71.00 $\pm$ 4.30 g/l. There were no significant differences between the pre-treatment and post-

infection values (Table 59). Similarly, there were no significant differences noted between the pre- and post-infection values of the untreated susceptible sheep which were infected with orf virus (Group 3) (Table 14). Comparing the mean values of the daily samples of the treated and untreated susceptible sheep revealed no significant differences (Table 60).

Estimations of the total serum proteins of the sera from eight previously infected sheep treated with the immunosuppressive drug before being re-infected with orf virus (group 13) ranged from 66 to 84 g/l in the sera taken before the treatment with betamethasone and from 62 to 79 g/l in the serum samples collected after treatment and challenge (Appendix Table 51).

The mean differences between the pre-treatment and post-challenge values were significantly lower from day 4 to day 12 (Table 61). In contrast, the mean differences between the pre - and post-challenge values of untreated sheep re-infected with orf virus (group 14) were not significant (Table 62). The total serum protein estimates of the untreated re-infected sheep ranged from 63 to 77 g/l in the pre-challenge sera and from 63 to 84 g/l in the post-challenge sera (Appendix Table 61). The comparison of the mean values of the daily samples from the treated and untreated re-infected sheep revealed that on days 6, 9, and 12 post-challenge there were significant differences (Table 63).

Specific serum protein levels: The electrophoretic evaluations of the serum proteins of the treated and untreated susceptible sheep infected with orf virus (groups 12 and 3) are shown in Appendix Tables 42-46, 22-26. The mean differences between the pre- and post-infection values of albumin and alpha-1, alpha 2-, beta-, and gammaglobulins of both the treated and the untreated susceptible sheep were not significant (Tables 64-68 15-19). There were no significant differences between the gammaglobulin daily means of the treated and the untreated sheep (Table 69 Figure 12).

Assessment of specific serum protein levels (Appendix Tables 52-56 62-66) in the sheep treated with betamethasone before re-infection (group 13) and the untreated but re-infected sheep (group 14) revealed that the mean differences between the pre- and post-challenge values of the albumin and alpha 1-, alpha 2-, and beta-globulins were not significant (Tables 70-77).

The mean differences for the gammaglobulin values, however, were significantly lower on days 2, 4, 6, 11, 12 and 13 after challenge of the treated re-infected sheep (Table 78). A plot of the daily means reflects the decreasing trend (Figure 13); the mean gammaglobulin values in the pre-treatment sera was 19.0 $\pm$ 2.5 g/l but following treatment with the immunosuppressive drug the values decreased in spite of challenge with orf virus. By the fourth day after challenge, the minimal mean levels re-

Figure 13 The daily means of gammaglobulin levels of:-

A - Previously infected sheep challenged with orf virus (Group 14).

B - Previously infected sheep treated with corticosteroid before being challenged with orf virus (Group 13).

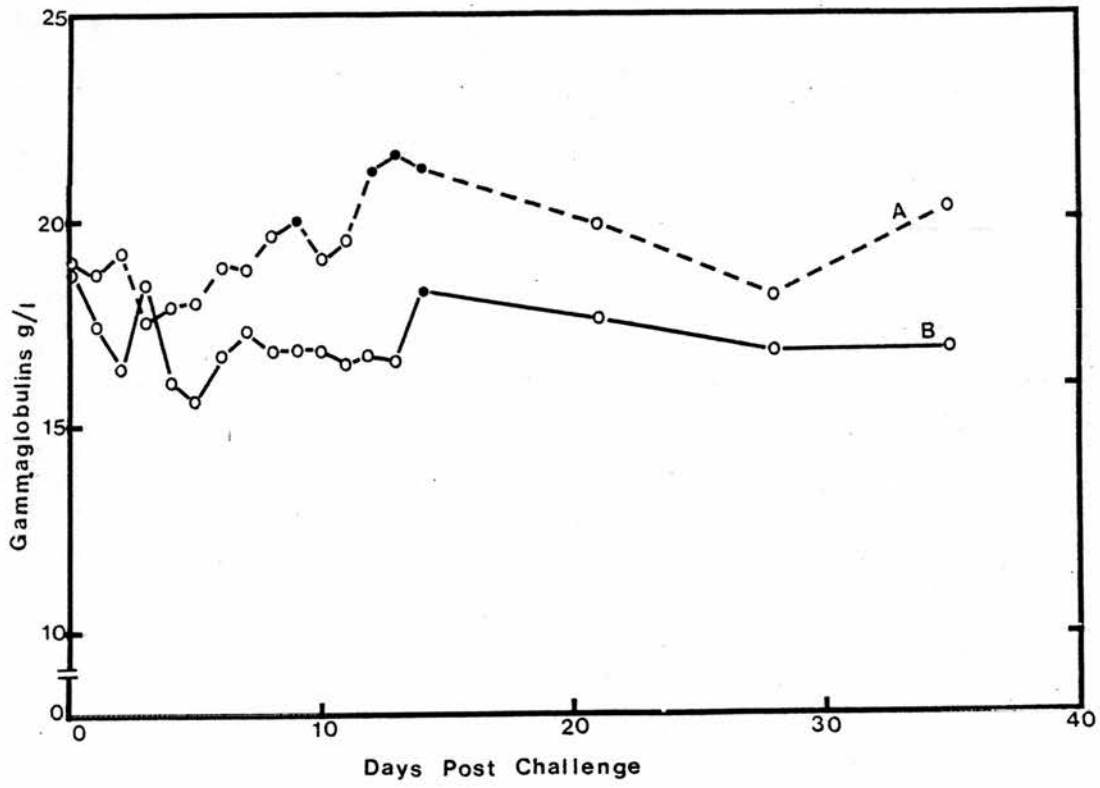
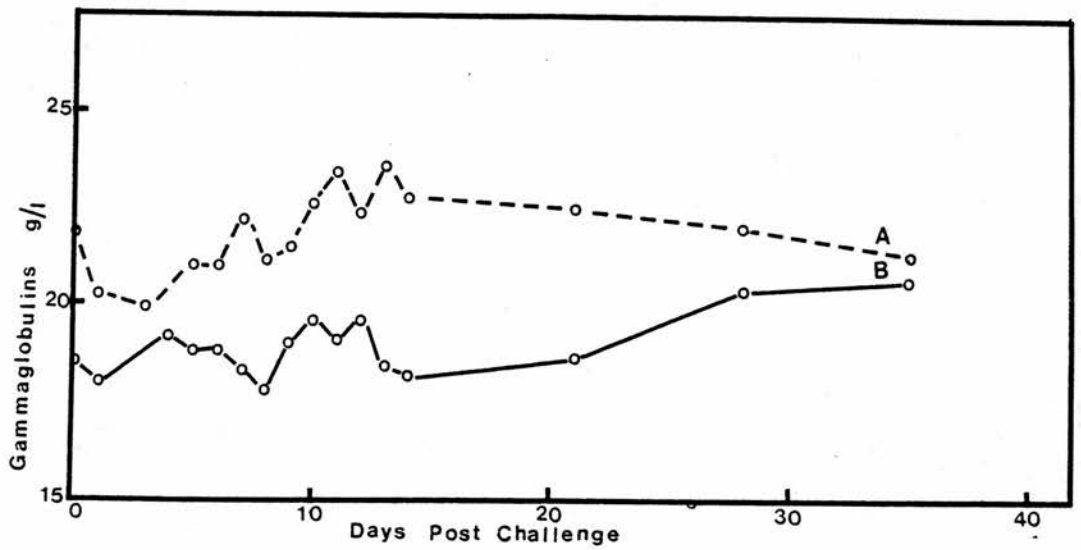


Figure 12 The daily means of gammaglobulin levels of:-

A - Susceptible sheep infected with orf virus (Group 3).

B - Susceptible sheep treated with corticosteroid before being infected with orf virus (Group 12).



corded was 16.6 ± 2.6 g/l. These low levels were maintained throughout until day 35, the last day of the experiment. Contrarily, the mean differences of gammaglobulin values between the pre- and post-challenge values of the untreated re-infected sheep (group 14) were not significant (Table 79) although the mean daily values showed an increasing trend (Figure 13).

Contrasting the daily mean values of gammaglobulins of treated re-infected sheep with the daily mean values of the untreated re-infected sheep revealed that on days 12 and 13 after challenge there were significant differences (Table 80).

Immunoglobulin concentrations: The levels of IgM, IgG1 and IgG2 in the pre- and post-challenge sera from eight susceptible sheep treated with betamethasone before being infected with orf virus (group 12) and of eight susceptible sheep also infected with orf virus (group 3) are shown in Appendix Tables 47-49/and plots of their daily mean values are illustrated in Figure 14.

27-29

The IgM levels of the treated susceptible sheep ranged from 1.2 to 4.7 g/l in the pre-treatment sera and 1.4 to 5.7 g/l in the post-challenge sera. The IgM mean values of the daily samples in the treated group 12 sheep ranged from 2.9 ± 1.1 g/l in the pre-treatment sera to 3.1 ± 1.3 g/l the highest value recorded after infection. The differences between pretreatment and post-infection values were not significant (Table 81). Similarly, the

Figure 14 The daily means of IgM, IgG_1 and IgG_2 levels in corticosteroid treated and untreated susceptible sheep infected with orf virus.

A - IgG_1 levels in untreated infected sheep (Group 3).

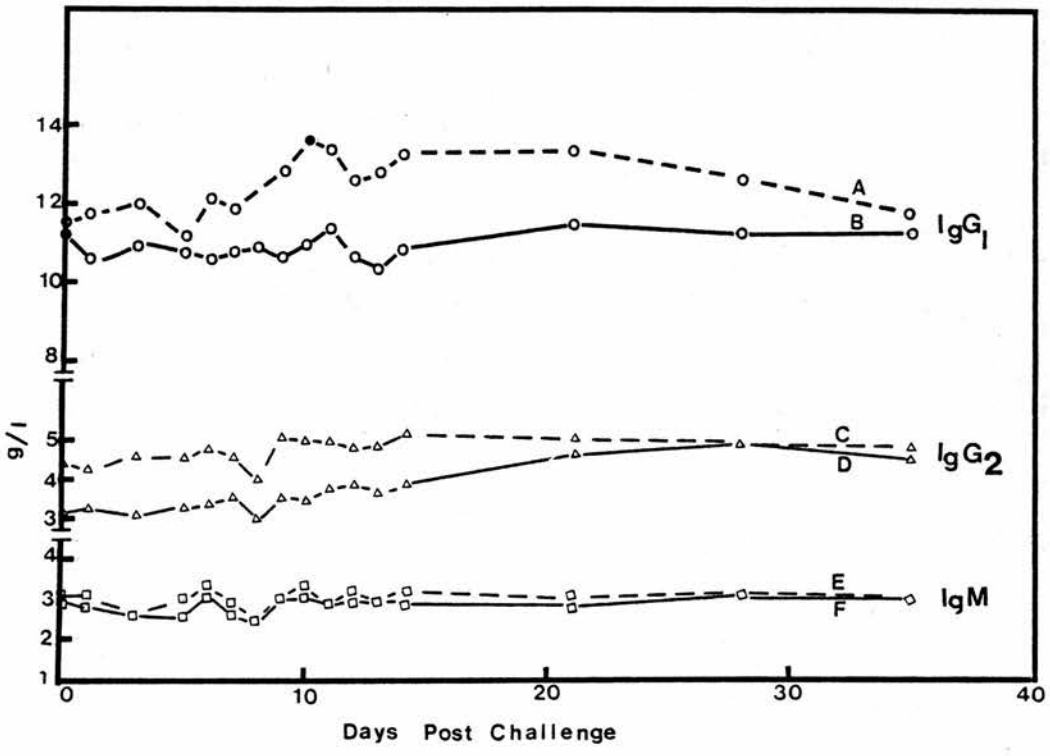
B - IgG_1 levels in treated infected sheep (Group 12).

C - IgG_2 levels in untreated infected sheep (Group 3).

D - IgG_2 levels in treated infected sheep (Group 12).

E - IgM levels in untreated infected sheep (Group 3).

F - IgM levels in untreated infected sheep (Group 12).


14
12
10
8
5
4
3
4
3
2
1
g/l
0
10
20
30
40
Days Post Challenge
A
B
C
D
E
F
IgG1
IgG2
IgM

differences between the pre- and post-infection values of the untreated sheep of group 3 were not significant (Table 30). The mean values of the daily samples of the untreated sheep of group 3 ranged from 3.0 ± 1.0 g/l in the pre-infection sera to 3.3 ± 1.0 g/l the highest value recorded on days 6 and 10 post-challenge. Comparison of the mean IgM values of treated and untreated susceptible sheep yielded no significant differences (Table 82).

The mean differences between the pre-treatment and post-infection values of IgG1 of the treated susceptible sheep of group 12 were not significant (Table 83). The mean values ranged from 11.25 ± 5.0 g/l in the pre-treatment sera and 11.50 ± 4.2 g/l, the highest values recorded on day 21 after infection. In contrast, the IgG1 levels of the untreated sheep of group 3 increased from 11.5 ± 5.9 g/l in the pre-infection sera to 13.6 ± 5.6 g/l on day ten after infection, and this increase was statistically significant (Table 31).

The IgG2 levels of the treated susceptible sheep ranged from 0.4 to 6.5 g/l in the pre-treatment sera and 0.9 to 7.5 g/l in the post-infections sera. The differences between the pre-treatment and post-infection values were significant on days 21, 28 and 35 after infection. (Table 84). UnLikewise, the differences between the pre- and post-infections values of the untreated susceptible sheep were not significant (Table 32).

Comparison of the daily means of IgG1 and IgG2 between the treated and the untreated susceptible sheep both infected with orf virus did not reveal any significant differences (Tables 85 and 86).

Changes in the levels of the IgM, IgG1 and IgG2 in the sera from eight previously infected sheep treated with betamethasone prior to challenge with orf virus (group 13) and from untreated eight previously infected sheep re-infected with orf virus (group 14) are illustrated in Figure 15 and the values are shown in Appendix Tables 57 - 59, and 67 - 69.

There were no significant changes in the IgM levels of the treated sheep of group 13 (Table 87), the mean values ranged from 4.2 $\pm$ 1.4 g/l in the pre-treatment sera to 4.4 $\pm$ 1.4 g/l recorded on day 11 after challenge. The IgM levels of the untreated sheep of group 14 also did not show significant change when the mean differences between the pre- and post-challenge values were calculated (Table 88). The mean values of the IgM of the untreated sheep of group 14 ranged from 3.7 $\pm$ 1.3 to 4.4 $\pm$ 1.4 g/l. No significant differences were revealed when the daily means of the IgM of the treated and the untreated re-infected sheep were compared (Table 89).

The IgG1 levels of the treated sheep of group 13 decreased significantly on the second and fourth day after challenge (Table 90). The mean IgG1 levels dropped from 12.3 $\pm$ 0.7 g/l in the pre-treatment sera to 9.3 $\pm$ 2.5 g/l in the sera collected after challenge, and then

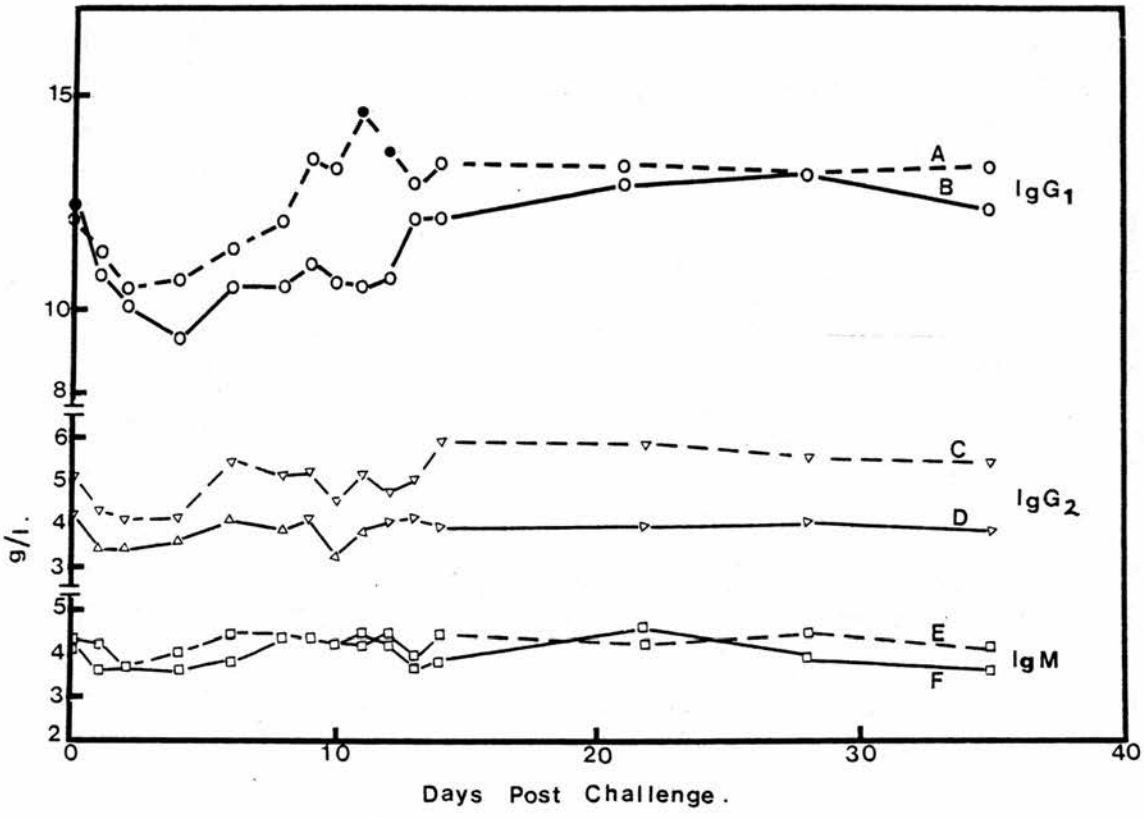


Figure 15: The daily means of the IgM, IgG_1 and IgG_2 levels in the corticosteroid treated and untreated previously infected sheep re-infected with orf virus.

A - IgG_1 levels in the untreated sheep (Group 14).

B - IgG_1 levels in the treated re-infected sheep (Group 13).

C - IgG_2 levels in the untreated reinfected sheep (Group 14).

D - IgG_2 levels in the treated re-infected sheep (Group 13).

E - IgM levels in the untreated re-infected sheep (Group 14).

F - IgM levels in the treated re-infected sheep (Group 13).

the levels returned to the pre-treatment values on the 13th day. In contrast, the IgG2 levels in the same serum samples increased significantly on days 12 through to 21 after challenge (Table 91). The mean values of the IgG2 in daily samples ranged from 3.2 $\pm$ 1.8 g/l in the pre-treatment sera to 4.1 $\pm$ 3,0 g/l on day 6 after challenge and then the levels were maintained at this level until day 35.

However, the IgG1 levels of the untreated sheep of group 14 rose significantly from 12.1 $\pm$ 2.7 g/l, mean value of the pre-challenge sera, to 14.6 $\pm$ 3.6 g/l, of mean value of samples collected on the 11th day after challenge, and gradually declined but the levels were still higher than the pre-challenge value 35 days after challenge (Table 92). Very slight changes were detected in the IgG_2 levels (Table 93).

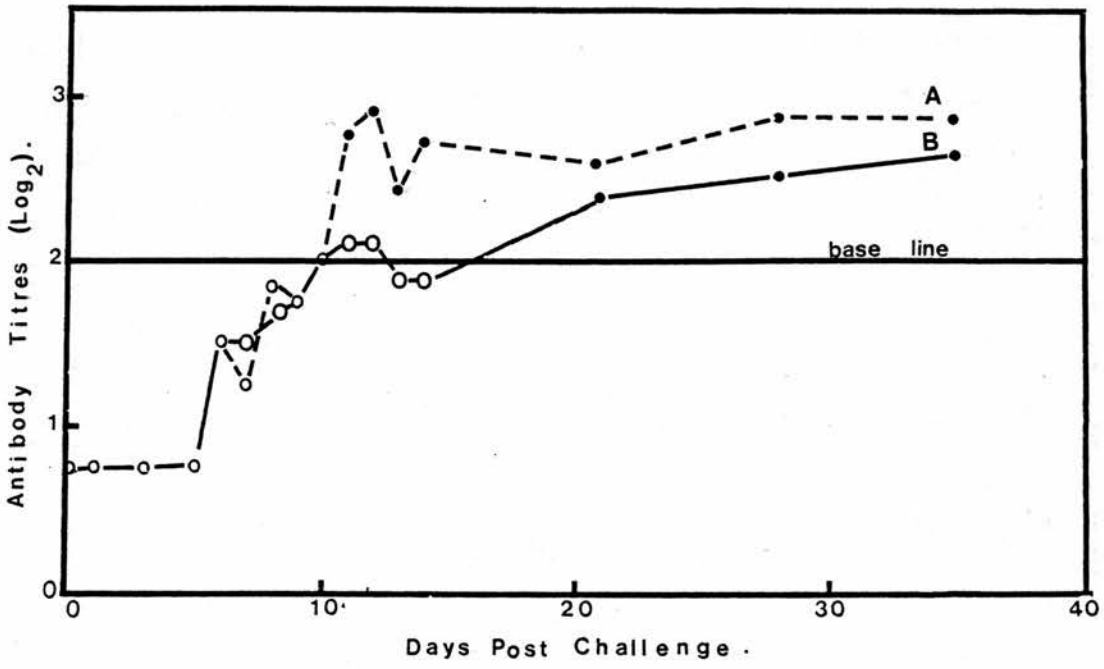
Comparison of the daily means of IgG1 and IgG2 between treated and the untreated re-infected sheep revealed significant differences only on day 11 post-challenge value for IgG1 (Tables 94 and 95).

Orf antibodies: There were no measurable orf antibodies in the pre-treatment sera from the eight susceptible sheep treated with betamethasone before infection with orf virus (group 12) and only minimal changes were observed in the first and second week after infection (Appendix Table 50). Antibodies started to appear in the third week rising gradually and were still rising in the fifth week when the experiment was terminated / (Table 96 & Figure 16). There were no correlations between the mean gammaglobulin values

Figure 16 The daily means of orf antibody titres of:-

A - Susceptible sheep infected with orf virus (Group 3).

B - Susceptible sheep treated with corticosteroid before being infected with orf virus (Group 12).



and the mean orf antibody titres in the same serum samples ($r_{(14)}$ = +0.49; $P > 0.05$) and between the mean IgG1 levels and mean antibody titres ($r_{(14)}$ = + 0.007; $P > 0.05$).

Similarly, the eight susceptible sheep not treated with the betamethasone (group 3) had no detectable orf antibodies in their pre-challenge sera and after infection changes were minimal during the first week but, thereafter, the titres rose to peak values in the fourth week after infection (Appendix Table 30).

Comparison of the daily mean antibody titres of the treated susceptible sheep of group 12 and those of the untreated susceptible sheep of group 3 revealed no significant differences (Table 97) but comparing the rate of antibody production between the two groups showed that the positions of the slopes of rate production were significantly different (F^1_{18} = 23.21; $P < 0.01$) (Figure 17). The slopes of the rate of antibody production were not significant (F^1_{18} = 3.16; $P > 0.05$).

None of the eight previously infected sheep treated with betamethasone before challenge with orf virus (group 13) had detectable orf antibodies in the sera collected before treatment. After treatment and challenge with the orf virus antibodies started to appear in the second week after challenge and reached peak titres five ~~three~~ weeks after challenge (Appendix Table 60, Table 98, Figure 18).

Correlation between the daily mean gammaglobulin values

Figure 17. The rate of orf antibody production in the corticosteroid treated and untreated susceptible sheep infected with orf virus.

A: Infected untreated susceptible sheep (Group 3).

B: Infected treated susceptible sheep (Group 12).

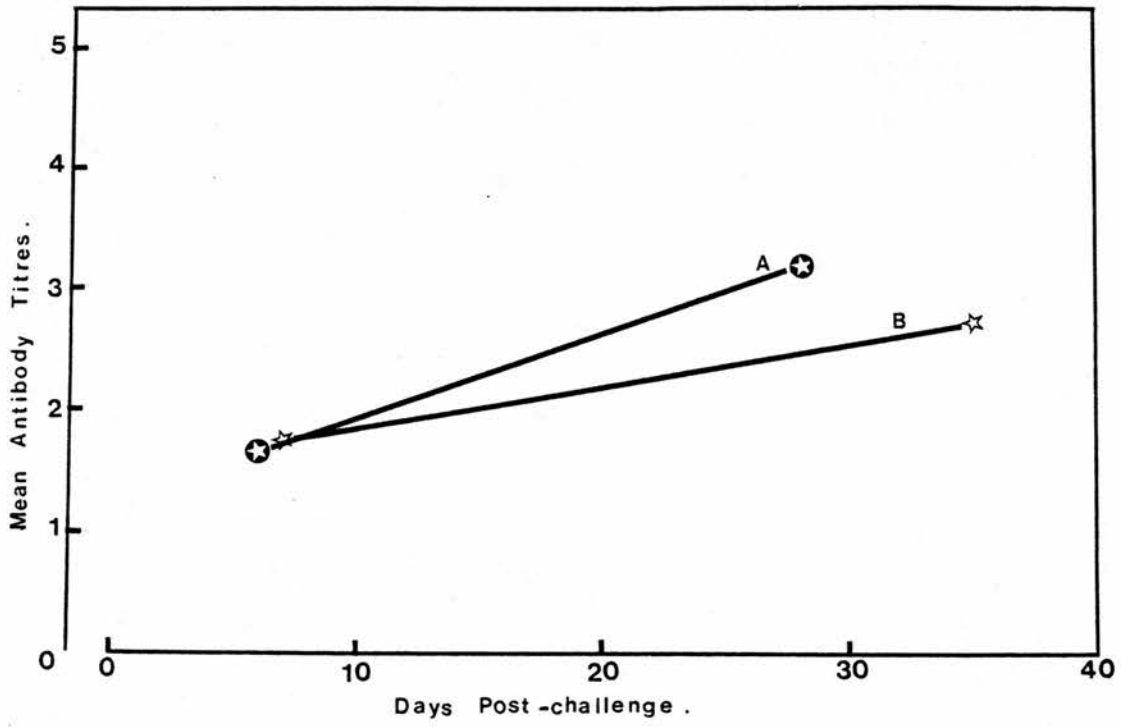
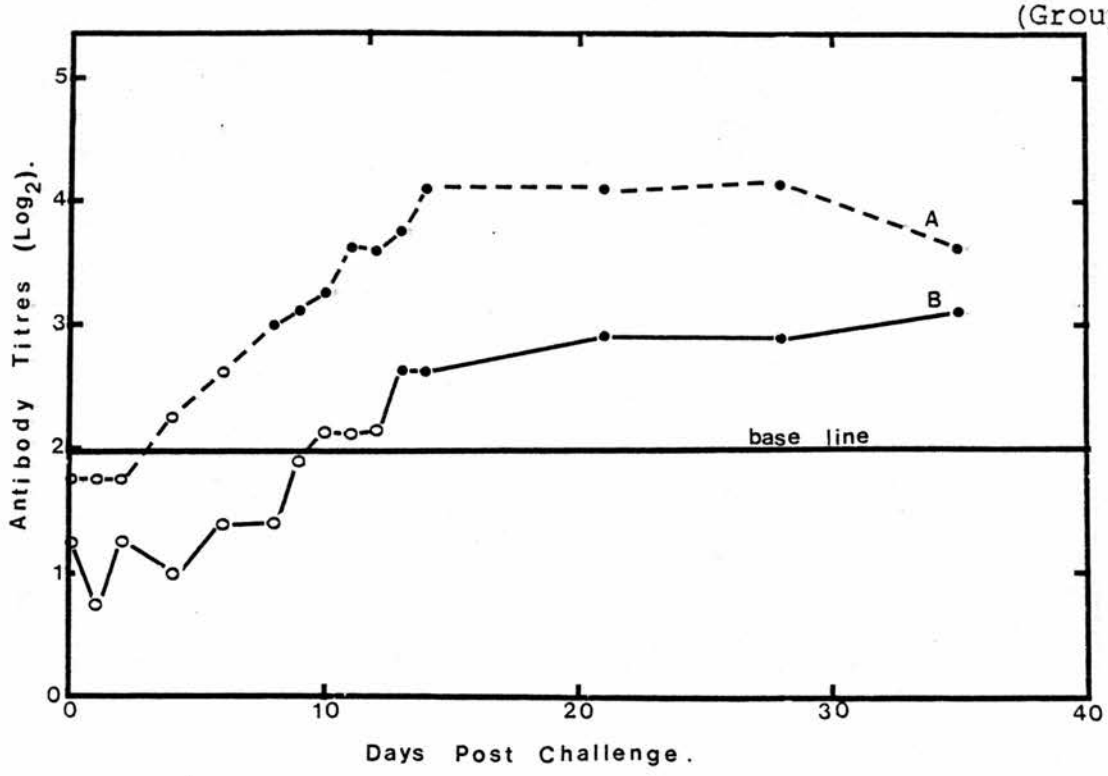


Figure 18 The daily means of orf antibody titres of:-

A - Previously infected sheep challenged with orf virus (Group 14)

B - Previously infected sheep treated with corticosteroid before being challenged with orf virus (Group 13).



and the daily mean orf antibody titres in the same serum sample were not significant ($r_{(14)}$ = + 0.06; $P > 0.05$). In contrast, the correlations of the daily means of IgG1 and IgG2 and the daily means of antibody titres were positive and significant ($r_{(13)}$ = + 0.64; $P < 0.01$ and $r_{(13)}$ = + 0.54; $P < 0.05$ respectively).

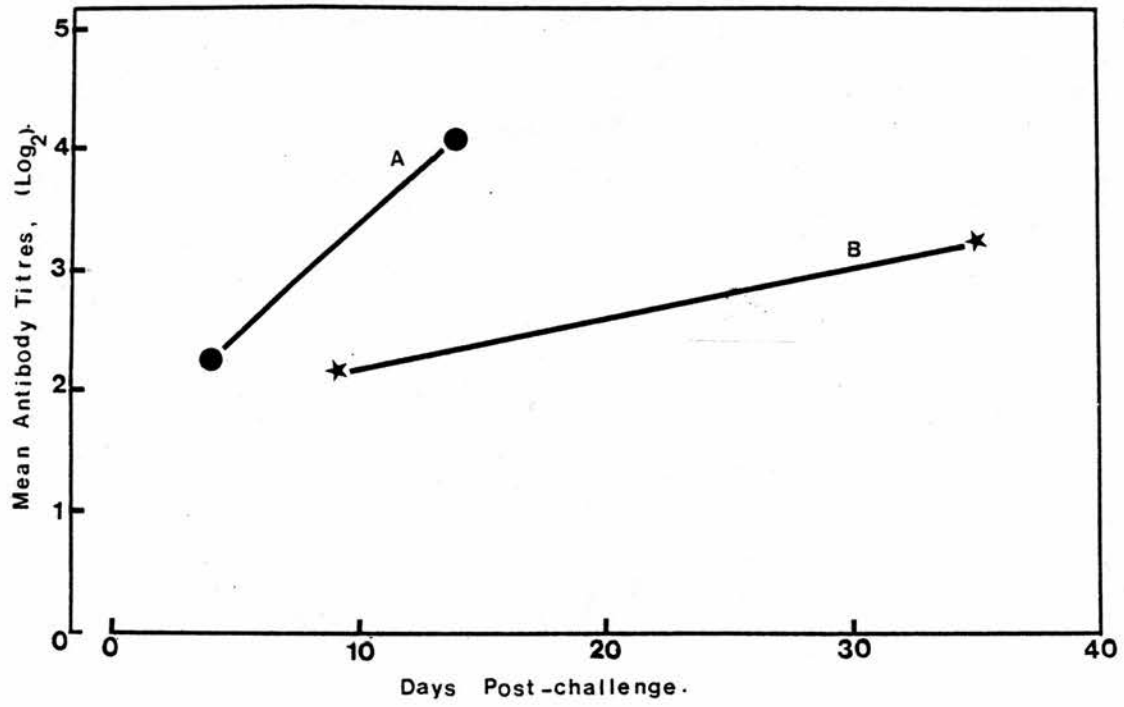
Two out of the eight previously infected sheep not treated with the immunosuppressive drug but challenged with orf virus (group 14) had detectable orf antibodies in the pre-challenge sera. After challenge, the antibody titres rose to reach a peak value in the second week which remained at this level in the third and fourth week before gradually declining (Appendix Table 70). Correlation of the daily mean values of the gammaglobulin and the mean antibody titres in the same serum samples was positive and significant ($r_{(14)}$= + 0.57; $P < 0.05$). Likewise, the correlations between the IgG1 values and the antibody titres was positive and significant ($r_{(14)}$= + 0.79; $P < 0.01$) (Table 99, Figure 18).

Comparing the mean orf antibody titres between the treated and the untreated previously infected sheep challenged with orf virus revealed a significant difference from days 4 to 28 after challenge (Table 100). The 'onset' of antibody production between the two groups were significantly slower in the treated sheep of group 13 and the peak values were reached much later too in group 13 sheep (Figure 19). The antibody production dynamics in the two groups of sheep were signifi-

Figure 19. The rate of orf antibody production in corticosteroid treated and untreated previously infected sheep reinfected with orf virus.

A - Untreated re-infected sheep (Group 14).

B - Treated re-infected sheep (Group 13).



cantly different ($r^{1}_{14} = 133.5$; $P < 0.01$).

When the rates of antibody production in the untreated previously infected sheep (group 14) and the untreated susceptible sheep of group 3 were compared, there were significant differences in the rate ($F^{2}_{23} = 7.90$; P 0.01) and the positions of the slopes ($F^{2}_{23} = 37.32$; P 0.01). Contrarily, when the rate of antibody production of the treated susceptible sheep and treated previously infected sheep were compared there were no significant differences in the slopes but the positions of the slopes differed significantly ($F^{1}_{16} = 0.45$; $P > 0.05$ and $F^{1}_{16} = 256.25$; $P < 0.01$ respectively) (Figure 20).

CMI Response Studies

The migratory index values for the eight susceptible sheep treated with betamethasone before being infected with orf virus were inconsistent (Appendix Table 34). The values varied from 98 percent in assays carried out before treatment and infection with the virus to 50 percent detected ten days after infection, then to 71 percent on day 14 and declined to 50 percent again on day 35, the last day of the experiment (Figure 21).

Comparing the results obtained from the infected susceptible sheep given immunosuppressive drug before infection with those from susceptible sheep infected without treatment revealed no significant difference between them (Table 101).

Figure 20: The rate of orf antibody production in sheep treated with corticosteroid before being infected or re-infected with orf virus.

A: Previously infected sheep (Group 13).

B: Susceptible sheep (Group 12).

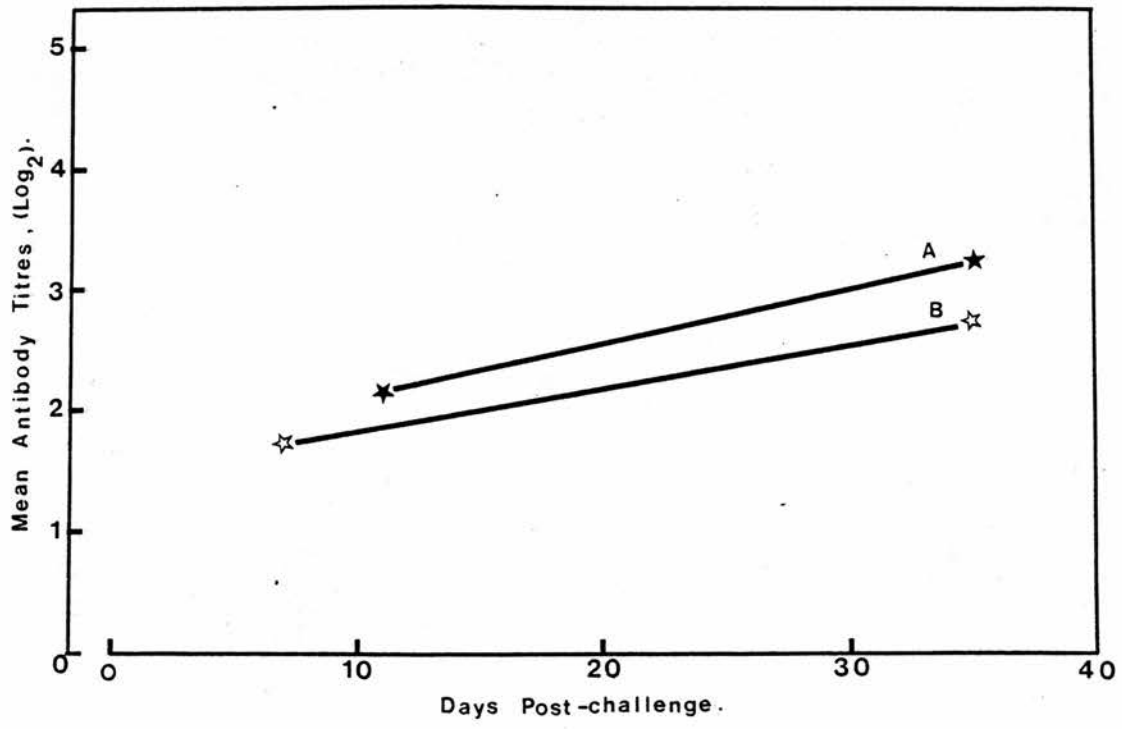


Figure 21 The daily means of the migration indices of:-

A - Susceptible sheep infected with orf virus (Group 3).

B - Susceptible sheep treated with corticosteroid before being challenged with orf virus (Group 12).

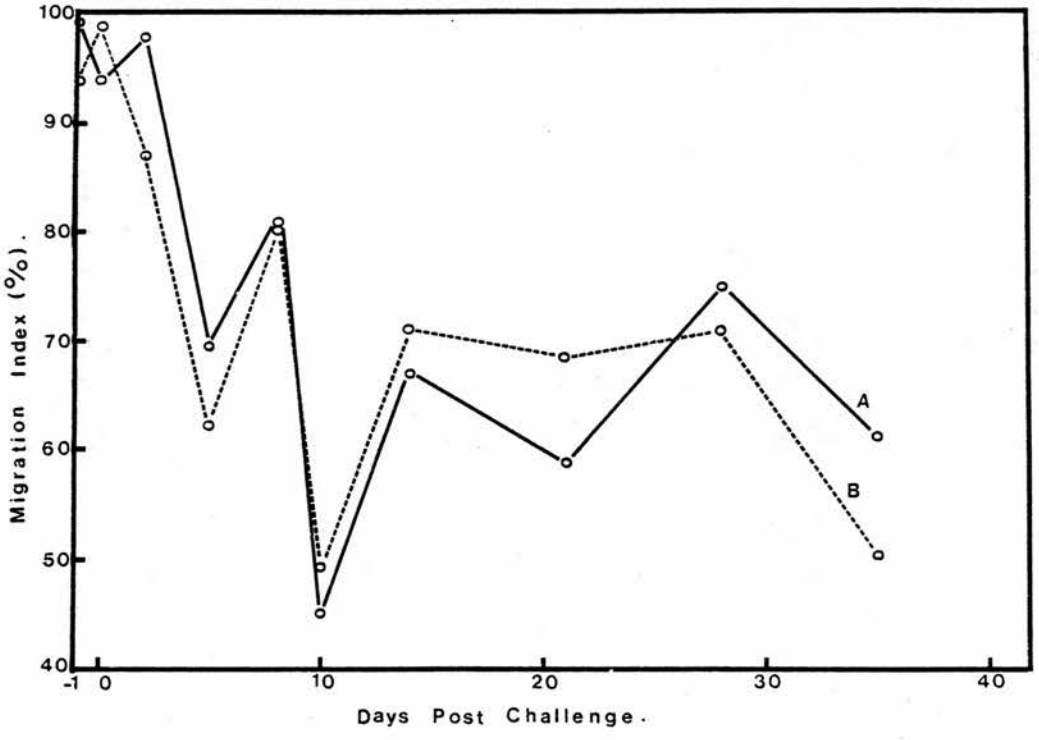


TABLE 59.

MEAN DIFFERENCES OF TOTAL SERUM PROTEIN CONTENTS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.50	0.63	0.79	> 0.50	N.S.
3	+ 2.25	2.45	0.92	> 0.40	N.S.
5	+ 2.87	1.47	1.96	> 0.10	N.S.
7	+ 3.75	0.94	3.99	< 0.01	S.
8	+ 2.25	1.01	2.22	> 0.10	N.S.
9	+ 1.00	2.03	0.49	> 0.70	N.S.
10	- 1.50	2.40	0.62	> 0.60	N.S.
11	- 1.12	2.18	0.52	> 0.70	N.S.
12	- 0.50	2.06	0.24	> 0.90	N.S.
13	0.00	1.75	0.00	> 0.90	N.S.
14	+ 1.37	1.85	0.74	> 0.50	N.S.
21	- 1.75	1.63	1.07	> 0.40	N.S.
28	- 1.75	2.00	0.87	> 0.50	N.S.
35	- 0.87	2.00	0.44	> 0.70	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 60.

COMPARISON OF THE DAILY MEANS OF TOTAL SERUM PROTEIN CONTENTS BETWEEN GROUPS 3 and 12.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	13	1.24	>0.30	N.S.
1	13	0.88	>0.40	N.S.
3	13	0.18	>0.90	N.S.
5	13	2.03	>0.10	N.S.
7	13	1.01	>0.40	N.S.
8	9	1.91	>0.10	N.S.
9	13	0.31	>0.80	N.S.
10	13	0.04	>0.90	N.S.
11	13	0.25	>0.90	N.S.
12	13	0.06	>0.90	N.S.
13	13	1.59	>0.20	N.S.
14	13	0.80	>0.50	N.S.
21	13	0.53	>0.70	N.S.
28	13	0.33	>0.80	N.S.
35	13	0.94	>0.40	N.S.

N.S. = Not Significant.

TABLE 61.

MEAN DIFFERENCE OF TOTAL SERUM PROTEIN CONTENTS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 5.00	1.39	3.60	< 0.01	H.S.
2	+ 4.00	2.02	1.98	> 0.10	N.S.
4	+ 5.50	1.69	3.25	< 0.02	S.
6	+ 8.00	2.12	3.77	< 0.01	H.S.
8	+ 6.37	1.74	3.66	< 0.01	H.S.
9	+ 6.37	2.84	2.84	< 0.05	S.
10	+ 7.00	2.86	2.44	< 0.05	S.
11	+ 6.75	2.44	2.77	< 0.05	S.
12	+ 5.00	1.91	2.62	< 0.05	S.
13	+ 6.00	2.69	2.23	> 0.10	N.S.
14	+ 3.75	2.35	1.60	> 0.20	N.S.
21	+ 5.62	2.14	2.63	< 0.05	S.
28	+ 5.75	2.76	2.03	> 0.10	N.S.
35	+ 7.37	2.32	3.17	< 0.02	S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 62.

MEAN DIFFERENCE OF TOTAL SERUM PROTEIN CONTENTS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 1.75	1.54	1.14	> 0.30	N.S.
2	- 1.37	1.00	1.37	> 0.30	N.S.
4	+ 0.50	1.51	0.33	> 0.80	N.S.
6	+ 1.37	1.41	0.97	> 0.40	N.S.
8	+ 2.12	2.11	1.00	> 0.40	N.S.
9	+ 1.25	1.63	0.77	> 0.50	N.S.
10	+ 0.20	2.06	0.10	> 0.90	N.S.
11	+ 1.75	1.93	0.91	> 0.40	N.S.
12	- 1.62	1.57	1.03	> 0.40	N.S.
13	+ 2.00	2.35	0.85	> 0.50	N.S.
14	- 1.37	1.88	0.73	> 0.50	N.S.
21	+ 1.62	2.05	0.79	> 0.50	N.S.
28	+ 1.50	1.84	0.81	> 0.50	N.S.
35	+ 0.71	1.23	0.58	> 0.60	N.S.

N.S. = Not Significant.

TABLE 63.

COMPARISON OF THE DAILY MEANS OF TOTAL SERUM PROTEIN CONTENTS BETWEEN GROUPS 13 and 14.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	14	0.76	> 0.5	N.S.
1	14	1.38	> 0.2	N.S.
2	14	1.11	> 0.3	N.S.
4	14	1.05	> 0.4	N.S.
6	14	2.27	< 0.05	S.
8	14	1.03	> 0.4	N.S.
9	14	2.51	< 0.05	S.
10	8	0.57	> 0.6	N.S.
11	14	1.61	> 0.2	N.S.
12	14	2.17	< 0.05	S.
13	14	0.49	> 0.7	N.S.
14	14	1.67	> 0.2	N.S.
21	14	1.24	> 0.3	N.S.
28	14	1.63	> 0.2	N.S.
35	14	1.63	> 0.2	N.S.

S. = Significant.

N.S. = Not Signicant.

TABLE 64.

MEAN DIFFERENCE OF ALBUMIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	- 0.56	0.82	0.68	> 0.600	N.S.
3	+ 3.02	1.62	1.87	> 0.100	N.S.
5	+ 3.92	1.27	3.09	< 0.020	S.
6	+ 4.27	0.41	10.43	< 0.001	H.S.
7	+ 2.76	1.44	1.92	> 0.100	N.S.
8	+ 1.79	1.03	1.73	> 0.200	N.S.
9	+ 2.04	1.55	1.31	> 0.300	N.S.
10	+ 0.56	1.56	0.36	> 0.800	N.S.
11	+ 1.09	0.38	2.86	< 0.050	S.
12	+ 2.41	2.31	1.04	> 0.040	N.S.
13	+ 2.94	1.83	1.60	> 0.200	N.S.
14	+ 1.24	1.39	0.89	> 0.400	N.S.
21	- 0.30	1.76	0.17	> 0.900	N.S.
28	+ 1.56	1.19	1.31	> 0.300	N.S.
35	+ 1.01	1.00	1.01	> 0.400	N.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 65.

MEAN DIFFERENCE OF ALPHA$_1$-GLOBULIN (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	- 0.32	0.29	1.12	> 0.30	N.S.
3	- 0.71	0.60	1.19	> 0.30	N.S.
5	- 0.75	0.49	1.53	> 0.20	N.S.
6	- 0.76	0.20	3.81	< 0.02	S.
7	- 0.47	0.42	1.13	> 0.30	N.S.
8	- 0.60	0.50	1.20	> 0.30	N.S.
9	- 0.77	0.53	1.46	> 0.20	N.S.
10	- 0.19	0.39	0.48	> 0.70	N.S.
11	- 0.27	0.59	0.47	> 0.70	N.S.
12	- 0.86	0.39	2.21	> 0.10	N.S.
13	- 0.46	0.40	1.16	> 0.30	N.S.
14	- 0.26	0.37	0.71	> 0.50	N.S.
21	+ 0.06	0.36	0.17	> 0.90	N.S.
28	+ 0.05	0.37	0.13	> 0.90	N.S.
35	+ 0.14	0.34	0.40	> 0.70	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 66.

MEAN DIFFERENCE OF ALPHA$_2$-GLOBULIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.56	0.56	1.00	>0.09	N.S.
3	- 0.25	0.83	0.30	>0.80	N.S.
5	- 0.01	0.65	0.02	>0.90	N.S.
6	- 0.20	0.56	1.16	>0.30	N.S.
7	- 1.26	1.18	1.07	>0.40	N.S.
8	+ 0.21	0.74	0.29	>0.80	N.S.
9	- 0.97	0.68	1.43	>0.20	N.S.
10	- 0.95	0.73	1.30	>0.30	N.S.
11	+ 0.24	0.62	0.38	>0.80	N.S.
12	- 0.70	0.79	0.89	>0.40	N.S.
13	- 1.14	0.81	1.40	>0.20	N.S.
14	- 0.59	0.60	0.98	>0.40	N.S.
21	- 1.05	0.78	1.35	>0.30	N.S.
28	- 0.89	0.71	1.25	>0.30	N.S.
35	- 1.15	0.92	1.25	>0.30	N.S.

N.S. = Not Significant.

TABLE 67.

MEAN DIFFERENCE OF BETA-GLOBULIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROIDS BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.01	0.25	0.05	> 0.90	N.S.
3	+ 0.37	0.52	0.72	> 0.50	N.S.
5	- 0.10	0.45	0.22	> 0.90	N.S.
6	+ 0.39	0.55	0.70	> 0.60	N.S.
7	- 0.61	0.54	1.13	> 0.30	N.S.
8	- 0.64	0.57	1.12	> 0.30	N.S.
9	- 0.76	0.46	1.66	> 0.20	N.S.
10	+ 0.06	0.49	0.13	> 0.90	N.S.
11	- 1.16	0.82	1.42	> 0.20	N.S.
12	- 1.36	0.76	1.79	> 0.20	N.S.
13	- 1.64	0.71	2.30	> 0.10	N.S.
14	- 0.06	0.69	0.09	> 0.90	N.S.
21	+ 0.20	0.51	0.39	> 0.80	N.S.
28	- 0.50	0.59	0.85	> 0.50	N.S.
35	- 0.29	0.34	0.84	> 0.50	N.S.

N.S. = Not Significant.

TABLE 68.

MEAN DIFFERENCE OF GAMMAGLOBULIN LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.50	0.80	0.63	>0.60	N.S.
3	+ 0.70	0.80	0.88	>0.50	N.S.
5	+ 0.23	0.80	0.29	>0.80	N.S.
6	+ 2.10	1.10	1.91	>0.10	N.S.
7	+ 0.20	0.70	0.29	>0.80	N.S.
8	+ 0.35	0.90	0.39	>0.80	N.S.
9	+ 0.55	0.90	0.61	>0.60	N.S.
10	+ 1.10	1.00	1.10	>0.40	N.S.
11	+ 0.60	0.70	0.86	>0.50	N.S.
12	+ 3.50	3.20	1.09	>0.40	N.S.
13	+ 0.10	0.90	0.11	>0.90	N.S.
14	+ 0.30	1.10	0.27	>0.80	N.S.
21	+ 0.20	1.30	0.15	>0.90	N.S.
28	+ 1.90	1.70	1.12	>0.30	N.S.
35	+ 2.15	2.00	1.08	>0.40	N.S.

N.S. = Not Significant.

TABLE 69.

COMPARISON OF THE DAILY MEANS OF GAMMAGLOBULIN LEVELS BETWEEN GROUPS 3 AND 12.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	13	1.43	> 0.20	N.S.
1	13	0.97	> 0.40	N.S.
3	13	0.27	> 0.80	N.S.
5	13	1.88	> 0.10	N.S.
6	5	0.42	> 0.70	N.S.
7	13	1.52	> 0.20	N.S.
8	9	1.36	> 0.20	N.S.
9	13	1.50	> 0.20	N.S.
10	13	1.09	> 0.30	N.S.
11	13	1.53	> 0.20	N.S.
12	13	1.01	> 0.40	N.S.
13	13	1.63	> 0.20	N.S.
14	13	1.53	> 0.20	N.S.
21	13	1.28	> 0.30	N.S.
28	13	0.58	> 0.60	N.S.
35	13	0.21	> 0.90	N.S.

N.S. = Not Significant.

TABLE 70.

MEAN DIFFERENCE OF ALBUMIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 4.07	2.27	1.79	>0.20	N.S.
2	+ 0.475	2.25	0.21	>0.90	N.S.
4	+ 0.425	2.47	0.17	>0.90	N.S.
6	+ 4.81	2.14	2.25	>0.10	N.S.
8	+ 3.83	1.65	2.32	<0.05	S.
9	+ 3.26	1.51	2.16	>0.10	N.S.
10	+ 3.64	2.27	1.60	>0.20	N.S.
11	+ 2.34	1.34	1.74	>0.20	N.S.
12	+ 2.29	1.30	1.76	>0.20	N.S.
13	+ 3.91	2.27	1.72	>0.20	N.S.
14	+ 3.17	2.31	1.37	>0.30	N.S.
21	+ 1.10	1.63	0.67	>0.60	N.S.
28	+ 0.78	2.17	0.36	>0.80	N.S.
35	+ 3.44	1.64	2.10	>0.10	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 71.

MEAN DIFFERENCE OF ALPHA$_1$-GLOBULIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.16	0.28	0.58	> 0.60	N.S.
2	+ 0.35	0.67	0.52	> 0.70	N.S.
4	+ 1.03	0.79	1.30	> 0.30	N.S.
6	+ 0.78	0.53	1.46	> 0.26	N.S.
8	+ 0.06	0.71	0.08	> 0.90	N.S.
9	+ 0.61	0.52	1.18	> 0.30	N.S.
10	+ 0.72	0.54	1.33	> 0.30	N.S.
11	+ 0.12	0.19	0.63	> 0.60	N.S.
12	+ 0.41	0.44	0.94	> 0.40	N.S.
13	+ 0.60	0.34	1.76	> 0.20	N.S.
14	+ 0.35	0.26	1.35	> 0.30	N.S.
21	+ 0.31	0.35	0.89	> 0.30	N.S.
28	+ 0.26	0.38	0.68	> 0.60	N.S.
35	+ 0.90	0.48	1.86	> 0.10	N.S.

N.S. = Not Significant.

TABLE 72.

MEAN DIFFERENCE OF ALPHA$_2$-GLOBULIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.42	0.56	0.76	> 0.50	N.S.
2	+ 1.30	0.57	2.28	> 0.10	N.S.
4	+ 0.87	0.55	1.58	> 0.20	N.S.
6	+ 1.26	0.75	1.68	> 0.20	N.S.
8	+ 0.82	0.68	1.20	> 0.30	N.S.
9	+ 1.29	0.78	1.65	> 0.20	N.S.
10	+ 1.22	0.78	1.56	> 0.20	N.S.
11	+ 0.71	0.60	1.18	> 0.20	N.S.
12	+ 0.80	0.50	1.60	> 0.20	N.S.
13	+ 0.57	0.53	1.08	> 0.40	N.S.
14	+ 0.74	0.43	1.71	> 0.20	N.S.
21	+ 1.16	0.99	1.17	> 0.30	N.S.
28	+ 1.15	0.81	1.42	> 0.20	N.S.
35	+ 0.52	0.55	0.95	> 0.40	N.S.

N.S. = Not Significant.

TABLE 73.

MEAN DIFFERENCE OF BETA-GLOBULIN LEVELS (DAY O - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.21	0.46	0.46	>0.70	N.S.
2	- 0.41	0.37	1.11	>0.40	N.S.
4	+ O.46	0.48	0.96	>0.40	N.S.
6	- 0.30	0.92	0.33	>0.80	N.S.
8	+ O.29	0.38	0.76	>0.50	N.S.
9	- 0.03	0.32	0.08	>0.90	N.S.
10	+ O.04	O.67	0.06	>0.90	N.S.
11	+ O.10	0.58	0.17	>0.90	N.S.
12	- 0.74	0.65	1.13	>0.30	N.S.
13	+ O.44	0.51	0.87	>0.50	N.S.
14	- O.83	0.66	1.25	>0.30	N.S.
21	+ O.30	0.55	0.54	>0.70	N.S.
28	+ O.20	0.33	0.60	>0.60	N.S.
35	+ O.44	0.32	1.37	>0.30	N.S.

N.S. = Not Significant.

TABLE 74.

MEAN DIFFERENCE OF ALBUMIN LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 1.41	1.06	1.33	>0.30	N.S.
2	- 0.49	1.89	0.26	>0.90	N.S.
4	- 1.73	1.89	0.91	>0.40	N.S.
6	+ 2.94	2.10	1.40	>0.20	N.S.
8	+ 3.74	2.43	1.54	>0.20	N.S.
9	+ 2.02	2.14	0.94	>0.40	N.S.
10	+ 0.96	2.81	0.34	>0.80	N.S.
11	- 0.20	2.31	0.09	>0.90	N.S.
12	+ 1.14	1.78	0.64	>0.60	N.S.
13	+ 4.87	2.92	1.67	>0.20	N.S.
14	+ 1.50	2.46	0.61	>0.60	N.S.
21	+ 0.74	2.83	0.26	>0.90	N.S.
28	+ 0.05	3.42	0.01	>0.90	N.S.
35	+ 1.47	2.86	0.51	>0.70	N.S.

N.S. = Not Significant

TABLE 75.

MEAN DIFFERENCE OF ALPHA$_1$-GLOBULIN LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.01	0.37	0.03	> 0.90	N.S.
2	- 0.78	0.62	1.25	> 0.30	N.S.
4	+ 0.24	0.50	0.48	> 0.70	N.S.
6	- 0.79	0.50	1.58	> 0.20	N.S.
8	- 0.59	0.34	1.73	> 0.20	N.S.
9	- 0.48	0.31	1.53	> 0.20	N.S.
10	+ 0.08	0.57	0.14	> 0.90	N.S.
11	+ 0.15	0.29	0.51	> 0.70	N.S.
12	- 0.13	0.42	0.31	> 0.80	N.S.
13	- 0.41	0.53	0.78	> 0.50	N.S.
14	- 0.75	0.51	1.47	> 0.20	N.S.
21	+ 0.25	0.38	0.66	> 0.60	N.S.
28	- 0.05	0.24	0.20	> 0.90	N.S.
35	- 0.36	0.63	0.57	> 0.60	N.S.

N.S. = Not Significant.

TABLE 76.

LEVELS

MEAN DIFFERENCE OF ALPHA$_2$-GLOBULIN/(DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.19	0.68	0.28	> 0.80	N.S.
2	+ 0.08	1.0	0.08	> 0.90	N.S.
4	+ 0.91	0.70	1.30	> 0.30	N.S.
6	+ 0.67	0.92	0.69	> 0.60	N.S.
8	+ 0.73	0.66	1.10	> 0.40	N.S.
9	- 0.14	0.79	0.17	> 0.90	N.S.
10	+ 0.78	0.90	0.87	> 0.50	N.S.
11	+ 1.79	0.67	2.67	< 0.05	S.
12	+ 0.56	0.92	0.61	> 0.60	N.S.
13	+ 0.71	1.19	0.60	> 0.60	N.S.
14	+ 0.50	0.81	0.62	> 0.60	N.S.
21	+ 0.71	0.68	1.04	> 0.40	N.S.
28	+ 1.26	0.96	1.31	> 0.30	N.S.
35	+ 0.14	1.11	0.13	> 0.90	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 77.

MEAN DIFFERENCE OF BETA-GLOBULIN LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.51	0.67	0.76	> 0.50	N.S.
2	- 0.25	0.77	0.32	> 0.80	N.S.
4	+ 0.14	1.01	0.14	> 0.90	N.S.
6	- 1.00	0.43	2.32	< 0.05	S.
8	- 0.87	0.50	1.75	> 0.20	N.S.
9	- 0.53	0.61	0.86	> 0.10	N.S.
10	+ 0.16	0.87	0.18	> 0.90	N.S.
11	+ 0.51	0.41	1.25	> 0.30	N.S.
12	- 0.10	0.57	0.17	> 0.90	N.S.
13	+ 0.21	0.60	0.35	> 0.80	N.S.
14	- 0.10	0.75	0.13	> 0.90	N.S.
21	- 0.88	0.88	1.00	> 0.40	N.S.
28	- 0.59	1.05	0.56	> 0.60	N.S.
35	- 0.01	0.75	0.02	> 0.90	N.S.

S. = Significant.

N.S.= Not Significant.

TABLE 78.

MEAN DIFFERENCE OF GAMMAGLOBULIN LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 1.55	1.05	1.48	> 0.20	N.S.
2	+ 2.60	0.62	4.15	< 0.01	H.S.
4	+ 3.00	1.10	2.73	< 0.05	S.
6	+ 2.30	1.00	2.30	< 0.05	S.
8	+ 2.20	1.10	2.00	> 0.10	N.S.
9	+ 2.20	1.10	2.00	> 0.10	N.S.
10	+ 2.90	1.50	1.93	> 0.10	N.S.
11	+ 2.50	0.90	2.78	< 0.02	S.
12	+ 2.25	0.95	2.35	< 0.05	S.
13	+ 2.40	0.92	2.61	< 0.05	S.
14	+ 1.14	1.29	0.88	> 0.50	N.S.
21	+ 1.37	0.80	1.72	> 0.20	N.S.
28	+ 2.15	1.20	1.79	> 0.20	N.S.
35	+ 2.60	1.40	1.86	> 0.10	N.S.

S. = Significant.
H.S. = Highly Significant.
N.S. = Not Significant.

TABLE 79.

MEAN DIFFERENCE OF GAMMAGLOBULIN LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	- 0.08	0.23	0.33	> 0.80	N.S.
2	- 0.35	1.10	0.32	> 0.80	N.S.
4	- 0.80	1.50	0.53	> 0.70	N.S.
6	- 0.15	0.50	0.30	> 0.80	N.S.
8	- 0.65	0.80	0.81	> 0.50	N.S.
9	- 1.30	1.10	1.18	> 0.30	N.S.
10	- 1.70	1.65	1.03	> 0.40	N.S.
11	- 0.70	1.00	0.70	> 0.60	N.S.
12	- 2.50	1.30	1.92	> 0.10	N.S.
13	- 2.90	1.50	1.92	> 0.10	N.S.
14	- 2.05	1.80	1.14	> 0.30	N.S.
21	- 0.60	1.00	0.60	> 0.60	N.S.
28	- 0.60	1.20	0.50	> 0.70	N.S.
35	- 2.30	1.70	1.35	> 0.30	N.S.

N.S. = Not Significant.

TABLE 80.

COMPARISON OF THE DAILY MEANS OF GAMMAGLOBULIN BETWEEN GROUPS 13 AND 14.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	14	0.19	> 0.90	N.S.
1	14	0.67	> 0.60	N.S.
2	14	1.64	> 0.20	N.S.
4	14	0.90	> 0.90	N.S.
6	14	1.47	> 0.20	N.S.
8	14	1.67	> 0.20	N.S.
9	14	1.73	> 0.20	N.S.
10	10	0.93	> 0.40	N.S.
11	14	1.59	> 0.20	N.S.
12	14	2.15	< 0.05	S.
13	14	2.76	< 0.02	S.
14	14	1.74	> 0.20	N.S.
21	14	1.28	> 0.30	N.S.
28	14	0.28	> 0.80	N.S.
35	13	1.46	> 0.20	N.S.

S. = Significant.

N.S. = N t Significant.

TABLE 81.

MEAN DIFFERENCE OF IgM LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	+ 0.13	0.22	0.57	>0.60	N.S.
3	+ 0.26	0.20	1.31	>0.30	N.S.
5	+ 0.31	0.20	1.56	>0.20	N.S.
6	- 0.07	0.35	0.21	>0.90	N.S.
7	+ 0.29	0.23	1.25	>0.30	N.S.
8	+ 0.12	0.11	1.06	>0.40	N.S.
9	- 0.09	0.20	0.44	>0.70	N.S.
10	- 0.16	0.27	0.60	>0.60	N.S.
11	+ 0.01	0.29	0.04	>0.90	N.S.
12	+ 0.06	0.20	0.31	>0.80	N.S.
13	+ 0.01	0.23	0.05	>0.90	N.S.
14	- 0.05	0.32	0.16	>0.90	N.S.
21	- 0.03	0.24	0.10	>0.90	N.S.
28	- 0.20	0.25	0.80	>0.50	N.S.
35	- 0.24	0.39	0.61	>0.60	N.S.

N.S. = Not Significant.

TABLE 82.

COMPARISON OF THE DAILY MEANS OF IgM LEVELS BETWEEN GROUPS 3 AND 12

Days Post-Challenge	Degrees of Freedom	t	P	Interpretation
0	13	0.28	>0.80	N.S.
1	13	0.41	>0.70	N.S.
3	13	0.63	>0.60	N.S.
5	13	0.63	>0.60	N.S.
6	5	0.17	>0.90	N.S.
7	13	0.68	>0.60	N.S.
8	9	0.29	>0.80	N.S.
9	13	0.16	>0.90	N.S.
10	13	0.34	>0.80	N.S.
11	13	0.17	>0.90	N.S.
12	13	0.36	>0.80	N.S.
13	13	0.17	>0.90	N.S.
14	13	0.35	>0.80	N.S.
21	13	0.43	>0.70	N.S.
28	13	0.21	>0.90	N.S.
35	13	0.21	>0.90	N.S.

N.S. = Not Significant.

TABLE 83.

MEAN DIFFERENCE OF IgG_1 LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.64	0.65	0.98	> 0.40	N.S.
3	+ 0.35	0.62	0.56	> 0.60	N.S.
5	+ 0.46	0.74	0.62	> 0.60	N.S.
6	+ 0.85	1.21	0.70	> 0.60	N.S.
7	+ 0.45	0.49	0.92	> 0.40	N.S.
8	+ 0.39	0.91	0.43	> 0.70	N.S.
9	+ 0.64	0.84	0.76	> 0.50	N.S.
10	- 0.15	0.58	0.26	> 0.90	N.S.
11	+ 0.17	1.14	0.15	> 0.90	N.S.
12	+ 0.56	0.93	0.60	> 0.60	N.S.
13	+ 0.81	0.86	0.94	> 0.40	N.S.
14	+ 0.40	0.93	0.43	> 0.70	N.S.
21	+ 0.24	1.27	0.19	> 0.90	N.S.
28	- 0.09	1.18	0.08	> 0.90	N.S.
35	- 0.16	1.12	0.14	> 0.90	N.S.

N.S. = Not Significant.

TABLE 84.

MEAN DIFFERENCE OF IgG_2 LEVELS (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.07	0.14	0.54	>0.70	N.S.
3	+ 0.07	0.21	0.36	>0.80	N.S.
5	- 0.07	0.20	0.37	>0.80	N.S.
6	- 0.37	0.29	1.29	>0.30	N.S.
7	- 0.41	0.24	1.72	>0.20	N.S.
8	- 0.53	0.44	1.21	>0.30	N.S.
9	- 0.41	0.34	1.21	>0.30	N.S.
10	- 0.35	0.31	1.13	>0.30	N.S.
11	- 0.40	0.34	1.76	>0.20	N.S.
12	- 0.80	0.33	2.24	>0.10	N.S.
13	- 0.51	0.45	1.14	>0.30	N.S.
14	- 0.76	0.43	1.77	>0.20	N.S.
21	- 1.51	0.36	4.20	<0.01	H.S.
28	- 1.83	0.55	3.32	<0.02	S.
35	- 1.66	0.56	2.97	<0.02	S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 85.

COMPARISON OF THE DAILY MEANS OF IgG_1 LEVELS BETWEEN GROUPS 3 and 12.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	13	0.09	> 0.90	N.S.
1	13	0.49	> 0.70	N.S.
3	13	0.44	> 0.70	N.S.
5	13	0.20	> 0.90	N.S.
6	5	0.41	> 0.70	N.S.
7	13	0.52	> 0.70	N.S.
8	9	0.53	> 0.70	N.S.
9	13	1.36	> 0.20	N.S.
10	13	1.05	> 0.40	N.S.
11	13	0.83	> 0.50	N.S.
12	13	0.82	> 0.50	N.S.
13	13	1.14	> 0.30	N.S.
14	13	1.23	> 0.30	N.S.
21	13	0.88	> 0.40	N.S.
28	13	0.97	> 0.40	N.S.
35	13	0.26	> 0.80	N.S.

N.S. = Not Significant.

TABLE 86.

COMPARISON OF THE DAILY MEANS OF IgG_2 LEVELS BETWEEN GROUPS 3 AND 12.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	13	1.18	> 0.30	N.S.
1	13	0.95	> 0.30	N.S.
3	13	1.58	> 0.20	N.S.
5	13	1.47	> 0.20	N.S.
6	5	0.84	> 0.50	N.S.
7	13	1.20	> 0.30	N.S.
8	9	1.47	> 0.20	N.S.
9	13	1.70	> 0.20	N.S.
10	13	1.61	> 0.20	N.S.
11	13	1.32	> 0.30	N.S.
12	13	0.97	> 0.40	N.S.
13	13	1.26	> 0.30	N.S.
14	13	1.35	> 0.20	N.S.
21	13	0.53	> 0.60	N.S.
28	13	0.00	> 0.00	N.S.
35	13	0.40	> 0.70	N.S.

N.S. = Not Significant.

TABLE 87.

MEAN DIFFERENCE OF IgM LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.88	0.70	1.26	> 0.30	N.S.
2	+ 1.09	0.69	1.58	> 0.20	N.S.
4	+ 1.04	0.70	1.49	> 0.20	N.S.
6	- 0.26	0.69	0.38	> 0.80	N.S.
8	+ 0.04	0.54	0.07	> 0.90	N.S.
9	- 0.09	0.40	0.22	> 0.90	N.S.
10	+ 0.35	0.55	0.64	> 0.60	N.S.
11	+ 0.06	0.28	0.22	> 0.90	N.S.
12	+ 0.49	0.41	1.19	> 0.30	N.S.
13	+ 0.15	0.65	0.23	> 0.90	N.S.
14	- 0.74	0.95	0.78	> 0.50	N.S.
21	- 0.23	0.72	0.31	> 0.80	N.S.
28	- 0.36	0.65	0.55	> 0.60	N.S.
35	- 0.21	0.72	0.29	> 0.80	N.S.

N.S. = Not Significant.

TABLE 88.

MEAN DIFFERENCE OF IgM LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.15	0.31	0.48	> 0.70	N.S.
2	+ 0.08	0.20	0.40	> 0.70	N.S.
4	+ 0.38	0.26	1.44	> 0.20	N.S.
6	- 0.06	0.37	0.17	> 0.90	N.S.
8	- 0.06	0.30	0.21	> 0.90	N.S.
9	+ 0.05	0.27	0.18	> 0.90	N.S.
10	+ 0.10	0.33	0.30	> 0.80	N.S.
11	+ 0.10	0.41	0.24	> 0.90	N.S.
12	- 0.01	0.50	0.03	> 0.90	N.S.
13	+ 0.41	0.39	1.06	> 0.40	N.S.
14	- 0.05	0.50	0.10	> 0.90	N.S.
21	+ 0.19	0.37	0.51	> 0.70	N.S.
28	- 0.09	0.63	0.14	> 0.90	N.S.
35	+ 0.29	0.44	0.65	> 0.60	N.S.

N.S. = Not Significant.

TABLE 89.

COMPARISON OF THE DAILY MEANS OF IgM LEVELS BETWEEN GROUPS 13 AND 14.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	14	0.19	> 0.90	N.S.
1	14	1.07	> 0.40	N.S.
2	14	0.17	> 0.90	N.S.
4	14	0.73	> 0.50	N.S.
6	14	0.91	> 0.40	N.S.
8	14	0.10	> 0.90	N.S.
9	14	0.00	> 0.90	N.S.
10	10	0.00	> 0.90	N.S.
11	14	0.34	> 0.80	N.S.
12	14	0.32	> 0.80	N.S.
13	14	0.81	> 0.50	N.S.
14	14	1.36	> 0.20	N.S.
21	14	0.48	> 0.70	N.S.
28	14	0.93	> 0.40	N.S.
35	13	0.23	> 0.90	N.S.

N.S. = Not Significant.

TABLE 90.

MEAN DIFFERENCE OF IgG_1 LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	p	Interpretation
1	+ 1.53	0.74	2.06	>0.10	N.S.
2	+ 2.25	0.58	3.88	<0.01	H.S.
4	+ 3.04	0.98	3.10	<0.02	S.
6	+ 1.05	0.82	1.28	>0.30	N.S.
8	+ 1.74	0.76	2.29	>0.10	N.S.
9	+ 0.69	0.49	1.41	>0.20	N.S.
10	+ 1.53	1.11	1.39	>0.20	N.S.
11	+ 1.86	0.79	2.35	<0.05	S.
12	+ 1.61	0.95	1.69	>0.20	N.S.
13	+ 0.24	0.66	0.36	>0.80	N.S.
14	+ 0.19	0.68	0.28	>0.80	N.S.
21	- 0.58	0.45	1.28	>0.30	N.S.
28	- 0.79	0.73	1.08	>0.40	N.S.
35	+ 0.09	0.60	0.15	>0.90	N.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 91.

MEAN DIFFERENCE OF IgG_2 LEVELS (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	- 0.20	0.18	1.11	> 0.30	N.S.
2	- 0.14	0.27	0.52	> 0.70	N.S.
4	- 0.45	0.21	2.14	> 0.10	N.S.
6	- 0.95	0.48	1.98	> 0.10	N.S.
8	- 0.66	0.33	2.01	> 0.10	N.S.
9	- 0.94	0.42	2.23	> 0.10	N.S.
10	- 0.40	0.44	0.91	> 0.40	N.S.
11	- 0.63	0.33	1.89	> 0.10	N.S.
12	- 0.81	0.34	2.39	< 0.05	S.
13	- 0.89	0.35	2.54	< 0.05	S.
14	- 0.74	0.35	2.11	> 0.11	N.S.
21	- 0.69	0.20	3.49	< 0.01	H.S.
28	- 0.84	0,44	1.90	> 0.01	N.S.
35	- 0.49	0.46	1.06	> 0.40	N.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 92.

MEAN DIFFERENCE OF IgG_1 LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.80	0.94	0.85	>0.50	N.S.
2	+ 1.54	1.10	1.40	>0.20	N.S.
4	+ 1.34	1.07	1.25	>0.30	N.S.
6	+ 0.68	1.14	0.59	>0.60	N.S.
8	- 0.05	0.90	0.05	>0.90	N.S.
9	- 1.51	0.82	1.84	>0.20	N.S.
10	- 1.22	1.60	0.76	>0.50	N.S.
11	- 2.53	0.90	2.80	<0.05	S.
12	- 2.33	0.90	2.60	<0.05	S.
13	- 1.29	0.92	1.40	>0.20	N.S.
14	- 1.35	0.79	1.71	>0.20	N.S.
21	- 1.26	1.17	1.08	>0.40	N.S.
28	- 1.10	0.54	2.04	>0.10	N.S.
35	- 1.18	1.14	1.04	>0.40	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 93.

MEAN DIFFERENCE OF IgG_2 LEVELS (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.51	0.41	1.24	> 0.30	N.S.
2	+ 0.40	0.41	0.98	> 0.40	N.S.
4	+ 1.10	0.29	3.93	> 0.01	H.S.
6	+ 0.26	0.33	0.80	> 0.40	N.S.
8	- 0.49	0.53	0.92	> 0.40	N.S.
9	+ 0.11	0.19	0.59	> 0.60	N.S.
10	+ 0.07	0.50	0.13	> 0.90	N.S.
11	+ 0.24	0.47	0.51	> 0.70	N.S.
12	+ 0.05	0.59	0.08	> 0.90	N.S.
13	+ 0.54	0.45	1.19	> 0.30	N.S.
14	+ 0.33	0.47	0.72	> 0.50	N.S.
21	+ 0.36	0.52	0.70	> 0.60	N.S.
28	+ 0.20	0.52	0.38	> 0.80	N.S.
35	+ 0.59	0.68	0.86	> 0.50	N.S.

N.S. = Not Significant.

TABLE 94.

COMPARISON OF THE DAILY MEANS OF IgG_1 BETWEEN GROUP 13 and 14

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	14	0.20	> 0.90	N.S.
1	14	0.49	> 0.70	N.S.
2	14	0.34	> 0.80	N.S.
4	14	1.08	> 0.30	N.S.
6	14	0.63	> 0.60	N.S.
8	14	1.63	> 0.20	N.S.
9	14	1.80	> 0.10	N.S.
10	10	1.10	> 0.30	N.S.
11	14	2.68	< 0.02	S.
12	14	1.68	> 0.20	N.S.
13	14	0.76	> 0.50	N.S.
14	14	1.35	> 0.20	N.S.
21	14	0.43	> 0.70	N.S.
28	14	0.12	> 0.90	N.S.
35	13	1.05	> 0.40	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 95.

COMPARISON OF DAILY MEANS OF IgG_2 BETWEEN GROUPS 13 AND 14

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	14	0.85	> 0.50	N.S.
1	14	0.82	> 0.50	N.S.
2	14	0.56	> 0.60	N.S.
4	14	0.64	> 0.60	N.S.
6	14	0.81	> 0.50	N.S.
8	14	1.07	> 0.40	N.S.
9	14	1.01	> 0.40	N.S.
10	10	0.98	> 0.40	N.S.
11	14	1.07	> 0.40	N.S.
12	14	0.62	> 0.60	N.S.
13	14	0.67	> 0.60	N.S.
14	14	1.28	> 0.30	N.S.
21	14	1.76	> 0.10	N.S.
28	14	1.30	> 0.30	N.S.
35	14	1.39	> 0.20	N.S.

N.S. = Not Significant.

TABLE 96.

MEAN DIFFERENCE OF ANTIBODY TITRES (DAY 0 - DAY X) OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Interpretation
1	0.00	0.00	0.00	> 0.90	N.S.
3	0.00	0.00	0.00	> 0.90	N.S.
5	+0.50	0.33	1.50	> 0.20	N.S.
6	-0.50	0.29	1.72	> 0.20	N.S.
7	-0.38	0.36	1.06	> 0.40	N.S.
8	-0.33	0.21	1.59	> 0.20	N.S.
9	-0.50	0.39	1.28	> 0.30	N.S.
10	-0.62	0.36	1.74	> 0.20	N.S.
11	-0.69	0.37	1.86	> 0.10	N.S.
12	-0.69	0.37	1.86	> 0.10	N.S.
13	-0.56	0.35	1.61	> 0.20	N.S.
14	-0.56	0.35	1.61	> 0.20	N.S.
21	-0.81	0.39	2.08	> 0.10	N.S.
28	-0.88	0.35	2.51	< 0.05	S.
35	-0.94	0.26	3.60	< 0.01	H.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 97.

COMPARISON OF THE DAILY MEAN ANTIBODY TITRES BETWEEN GROUPS 3 AND 12

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	13	0.28	> 0.8	N.S.
1	13	0.28	> 0.80	N.S.
3	13	0.28	> 0.80	N.S.
5	13	1.32	> 0.30	N.S.
6	5	0.00	> 0.00	N.S.
7	13	0.70	> 0.50	N.S.
8	9	0.14	> 0.90	N.S.
9	13	0.45	> 0.70	N.S.
10	13	0.50	> 0.70	N.S.
11	13	1.07	> 0.30	N.S.
12	13	1.43	> 0.20	N.S.
13	13	2.35	< 0.05	S.
14	13	2.03	> 0.10	N.S.
21	13	0.59	> 0.60	N.S.
28	13	0.84	> 0.50	N.S.
35	13	0.65	> 0.50	N.S.

S = Significant.

H.S. = Not Significant.

TABLE 98.

MEAN DIFFERENCE OF ORF ANTIBODIES (DAY 0 - DAY X) OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	+ 0.50	0.32	1.50	> 0.30	N.S.
2	0.00	0.00	0.00	> 0.90	N.S.
4	+ 0.25	0.59	0.42	> 0.70	N.S.
6	- 0.13	0.61	0.20	> 0.90	N.S.
8	- 0.13	0.61	0.20	> 0.90	N.S.
9	- 0.63	0.42	1.50	> 0.30	N.S.
10	- 0.88	0.58	1.51	> 0.30	N.S.
11	- 0.88	0.58	1.51	> 0.30	N.S.
12	- 0.71	0.39	1.82	> 0.20	N.S.
13	- 1.38	0.53	2.59	< 0.05	S.
14	- 1.38	0.42	3.30	< 0.02	S.
21	- 1.63	0.37	4.39	< 0.01	H.S.
28	- 1.63	0.42	3.87	< 0.01	H.S.
35	- 1.88	0.58	3.23	< 0.02	S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 99.

MEAN DIFFERENCE OF ANTIBODY TITRES (DAY 0 - DAY X) OF SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge (X)	Mean Difference	Standard Error	t	P	Inter-pretation
1	0.00	0.00	0.00	> 0.900	N.S.
2	0.00	0.00	0.00	> 0.900	N.S.
4	- 0.50	0.32	1.56	> 0.20	N.S.
6	- 0.88	0.29	3.01	< 0.020	S.
8	- 1.25	0.31	4.03	< 0.010	H.S.
9	- 1.38	0.37	3.72	< 0.010	H.S.
10	- 1.50	0.32	4.69	< 0.010	H.S.
11	- 1.88	0.40	4.69	< 0.010	H.S.
12	- 1.88	0.40	4.69	< 0.010	H.S.
13	- 2.00	0.38	5.26	< 0.001	H.S.
14	- 2.40	0.37	6.49	< 0.001	H.S.
21	- 2.40	0.37	6.49	< 0.001	H.S.
28	- 2.40	0.37	6.49	< 0.001	H.S.
35	- 1.88	0.35	5.36	< 0.001	H.S.

S. = Significant.

H.S. = Highly Significant.

N.S. = Not Significant.

TABLE 100.

COMPARISON OF THE DAILY MEANS ANTIBODY TITRES BETWEEN GROUPS 13 AND 14.

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
0	14	1.82	> 0.10	N.S.
1	14	1.84	> 0.10	N.S.
2	14	0.91	> 0.40	N.S.
4	14	3.04	< 0.01	S.
6	14	2.52	< 0.05	S.
8	14	3.26	< 0.01	S.
9	14	2.19	< 0.05	S.
10	14	2.11	> 0.10	N.S.
11	14	2.64	< 0.02	S.
12	14	3.22	< 0.01	S.
13	14	2.70	< 0.02	S.
14	14	3.10	< 0.01	S.
21	14	3.91	< 0.01	S.
28	14	3.37	< 0.01	S.
35	14	1.05	> 0.40	N.S.

S. = Significant.

N.S. = Not Significant.

TABLE 101.

COMPARISON OF THE DAILY MEANS OF MIGRATORY INDICES BETWEEN THE TREATED AND UNTREATED SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS (3 & 12)

Days Post-Challenge	Degrees of Freedom	t	P	Inter-pretation
- 2 before treatment	14	1.40	>0.20	N.S.
- 1 after treatment	10	1.01	>0.40	N.S.
2	14	0.60	>0.60	N.S.
5	10	0.44	>0.70	N.S.
8	14	0.08	>0.90	N.S.
10	2	0.10	>0.90	N.S.
14	13	0.57	>0.60	N.S.
21	13	0.43	>0.70	N.S.
28	9	0.40	>0.70	N.S.
35	12	1.21	>0.30	N.S.

N.S. = Not Significant.

DISCUSSION

My explorative study of the effects of immunosuppression on orf, summarized in Table 102, showed that corticosteroid treatment delayed the onset of the lesions and prolonged the course of the lesions in the treated sheep compared to the untreated sheep irrespective of whether the sheep was susceptible or previously infected. The postponement of the onset of the lesions and the subsequent prolonged course were attributable to anti-inflammatory effects and immunosuppressive activity of the corticosteroids (Nathanson and Cole 1971; Narita, et al., 1979). Complete healing in the treated susceptible sheep occurred in the sixth week after infection compared to the four weeks taken by the untreated infected susceptible sheep. Similarly, in the treated re-infected sheep healing occurred in the third week in contrast to the two weeks taken by the untreated re-infected sheep. The findings support Glasgow (1970) who reported that immunosuppressive drugs enhanced poxvirus virulence and Osman (1976) who found that corticosteroid therapy before challenge caused sheep to react severely to orf virus. Shope, Muscaplat, Chen and Johnson (1976), similarly, observed that dexamethasone treated calves with no detectable neutralizing antibodies to bovine viral diarrhoea virus developed a fatal viraemia while the untreated did not.

TABLE 102.

SUMMARY OF RESULTS FROM EXPLORATORY STUDY OF THE EFFECTS OF IMMUNOSUPPRESSION ON ORF INFECTIONS.

Measured	SUSCEPTIBLE SHEEP		PREVIOUSLY INFECTED SHEEP	
	Treated	Untreated	Treated	Untreated
Total serum Protein	No change	As expected	Depressed	As expected
Gamma-globulin	No change	No change	Depressed	As expected
IgM	No change	No change	No change	As expected
IgG_1	No change	As expected	Depressed	As expected
IgG_2	Raised	As expected	Raised	As expected
Orf Antibodies:				
Titres	Lower	As expected	Lower	As expected
Rate	Slower	As expected	Slower	As expected
CMI Response	Present	Present	Not studied	Not studied
Lesion:				
Onset	Slower	As expected	Slower	As expected
Course	Longer	As expected	Longer	As expected

The prolonged course of the lesions in the treated susceptible sheep was mirrored by the slower onset of detectable antibodies which reached lower peak titres at much later time compared to the untreated infected susceptible sheep. The expected drops in the IgM and IgG1 levels of the treated infected susceptible sheep were not observed and although slight insignificant increases were detected in the untreated infected susceptible sheep, the increases were not significantly different from the levels of the treated infected susceptible sheep. Significant increases were detected in the IgG2 levels in both the treated and the untreated infected susceptible sheep. This might be due to the lymphocytes responsible for producing IgG2 being resistant to corticosteroid toxicity.

In the treated re-infected sheep depression of the gammaglobulin and IgG1 levels were observed while in the untreated re-infected sheep the gammaglobulin and IgG1 levels were raised as expected. The rate of antibody production, likewise, was significantly slower in the treated re-infected sheep compared to the untreated re-infected sheep. In contrast, the expected drop in the IgG2 levels in the treated re-infected sheep was not observed instead a significant raise was detected which was not observed in the untreated re-infected sheep. A likely explanation here might again be the selective resistance of lymphocytes to corticosteroid toxicity. As

expected no changes were observed in the IgM levels of the treated and the untreated re-infected sheep.

The present findings are in agreement with the observation made by Zurier and Weissman (1973) that the induction phase of antibody production was more prone to steroid suppression. However, my results diverge from their concept that primary antigen responseswere more vulnerable to steroid interference than secondary antigen response. My data indicated that humoral immune responses were suppressed in both primary and secondary infections although much more obvious in the secondary infections. A tentative explanation here could be that the effects of the corticosteroid in both primary and secondary reactions were the same and that the effects in secondary reactions were more obvious because the humoral immune responses in secondary reactions were also more pronounced. On the other hand, the one dose treatment of the immunosuppressive drug used in my study might not have been effective enough for susceptible sheep especially the course of orf being much longer in primary infections, by the time the virus multiplication in the lesions maximise, the effects of the corticosteroid might have worn off.

The corticosteroid did not seem to have any effect on the CMI responses because the LMI tests performed in the treated susceptible sheep infected with orf virus gave similar positive indications to those performed in the untreated susceptible sheep also infected with orf

virus. I failed to find any information in the literature on the effects of corticosteroids on CMI in sheep but Davies and Carmichael (1973) reported that in cattle which were infected with infectious bovine rhinotracheitis virus and three months later treated with dexamethasone for five days, the CMI, as measured by lymphocyte transformation tests, was suppressed during the time of recrudescence of the infection. Again a likely explanation for my findings might be the fact that the one dose-treatment was not enough to suppress CMI responses. Earlier Claman (1972) had also observed that even in the steroid-resistant animals the CMI manifestations could be suppressed when large doses or prolonged treatment were carried out. In addition, my experiments are open to criticisms in that I did not investigate CMI in treated previously infected sheep. It will be interesting to examine the effects of corticosteroid on CMI responses in treated previously infected sheep especially because I found that CMI played a great role in the abrupt clearance of the virus in the accelerated reactions.

Unfortunately, in these experiments control groups of sheep treated with corticosteroid without infection or re-infection were not included. In addition, surveys carried out in other species, suggest that there is a species heterogeneity in the response of lymphoid cells and their immunologic reactions to corticosteroids and remarkable heterogeneity within the lymphoid cells compartment even in a given species. I venture to suggest

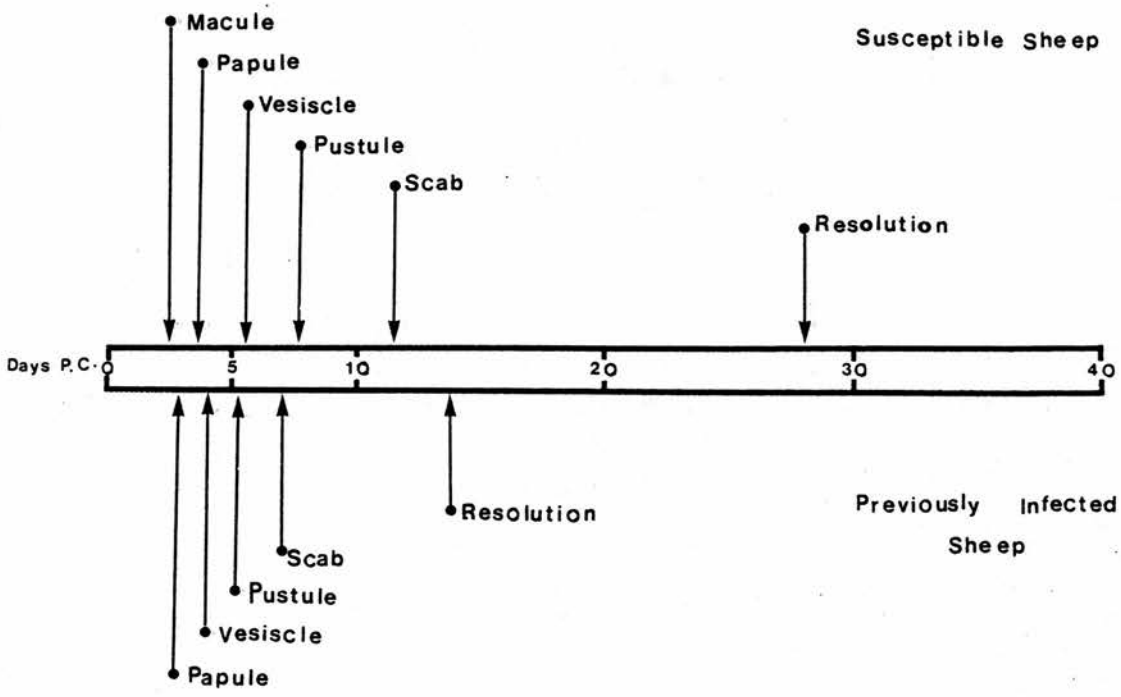
that another form of immunosuppression should be used and examination of the growth of the virus in the immunosuppressed sheep be carried out at the same time. Nevertheless, my results point to humoral factors as being important in the recovery from orf virus infections.

CHAPTER SEVEN

GENERAL DISCUSSION

When suspensions of orf scabs were applied to freshly scarified skins of susceptible sheep of any age, prominent orf lesions that evolved through the classical pox stages of macule, papule, vesicle, pustule and scab resulted. Likewise, when orf scab suspensions were applied to freshly scarified skins of previously infected sheep, the resulting lesions progressed very rapidly through the typical pox stages, healing occurring within three weeks (Boughton and Hardy, 1934; Hart et al., 1949; Schmidt, 1962; Osman, 1976) (Figure 22). Osman (1976) likened the phenomenan to the well-known accelerated immune response that occurs in man re-vaccinated against smallpox. In addition, he found that virus replication occurred in both the primary and the accelerated reactions and that the growth curves of the virus in both infected and re-infected sheep, as measured by the number of virus particles in the epidermal layers of the scarified skin, was essentially the same initially. He showed that the number of virus particles in the epidermal layers of scarified skin of susceptible sheep rose exponentially to reach maximum titres in four days then levelled for a period of three weeks before it declined gradually over a period of another three weeks. The number of virus particles in the scarified skins of previously infected sheep also rose exponentially reaching a maximum five days after re-infection but the titre was significantly lower than in the susceptible sheep. The titre was only

Figure 22 Onsets of the lesion stages in experimentally infected and re-infected sheep.



maintained at this level for three days when it decreased abruptly (Figure 23).

Glover (1933) believed that sheep recovering from orf virus infections developed a 'solid' immunity. Osman (1976) and I found that recovered sheep reacted when challenged with the virus despite the presence of detectable humoral antibodies.

Romero-Mercado (1969) was the first to link the appearance of scabs in both primary and accelerated orf reactions to the development of demonstrable humoral antibodies. An examination of my data confirms this relationship of the lesion evolution and humoral immune response in both primary and accelerated reactions such that the onset of the scab stage coincided with the appearance of humoral antibodies and the resolution of the lesions was associated with the occurrence of peak antibody titre (Figures 24 and 25).

Furthermore, when my findings were related to those of Osman (1976) on the growth curves of the virus in primary reactions in susceptible sheep, the appearance of detectable humoral antibodies was found to coincide with the onset of the exponential decline of the virus titre. A more dramatic relationship was noted when my data on the humoral immune responses in re-infected sheep was compared with the growth pattern of the virus in previously infected sheep (Osman 1976). The exponential increase of the antibody titre coincided with the abrupt disappearance of the virus. Hence, there is an implication

Figure 23 Growth curves of orf virus in the skins of susceptible and previously infected sheep. Redrawn with permission from Dr. Omar A.H. Osman's thesis (1976).

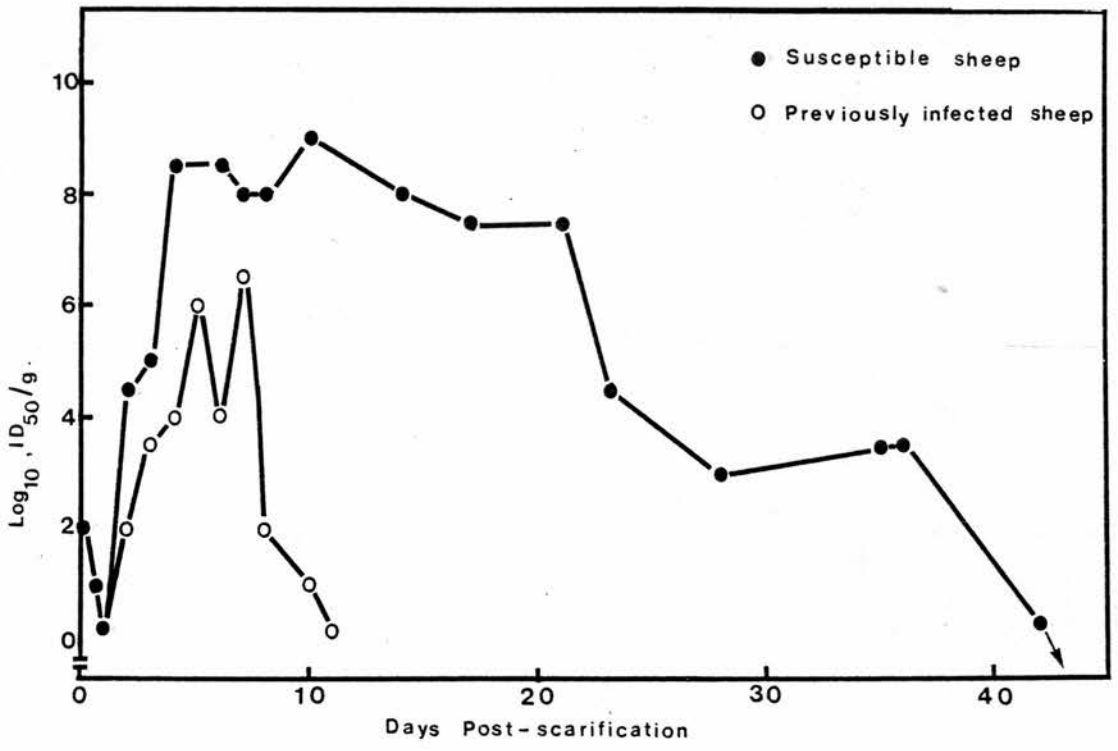


Figure 24 Relationship between orf lesion stages and antibody titres in infected susceptible sheep.

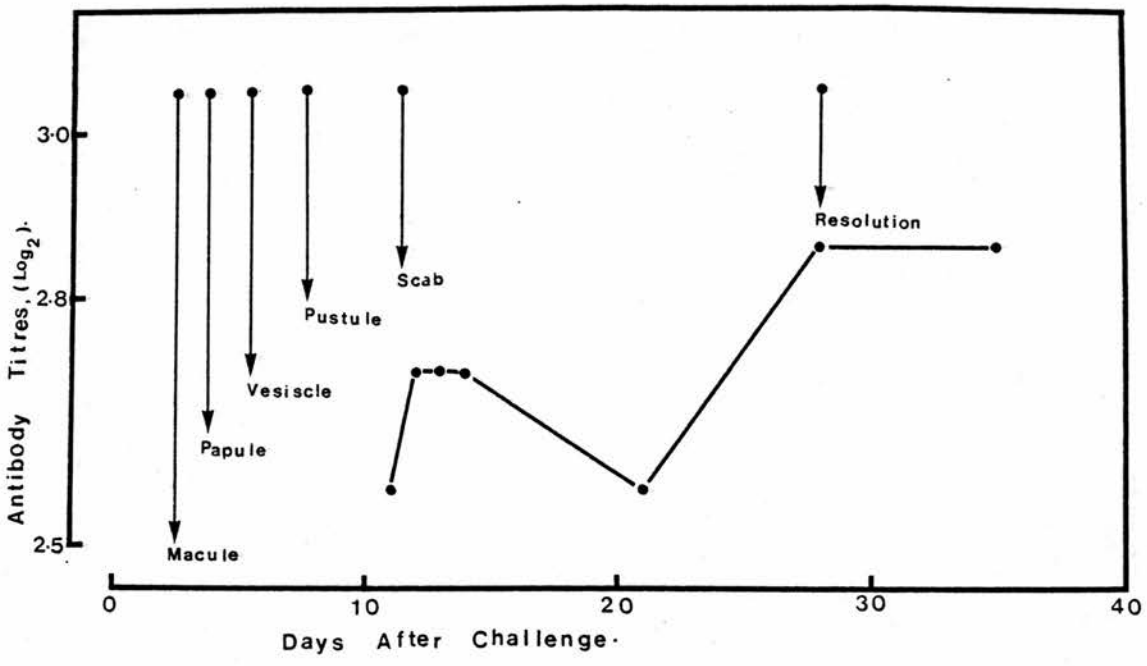
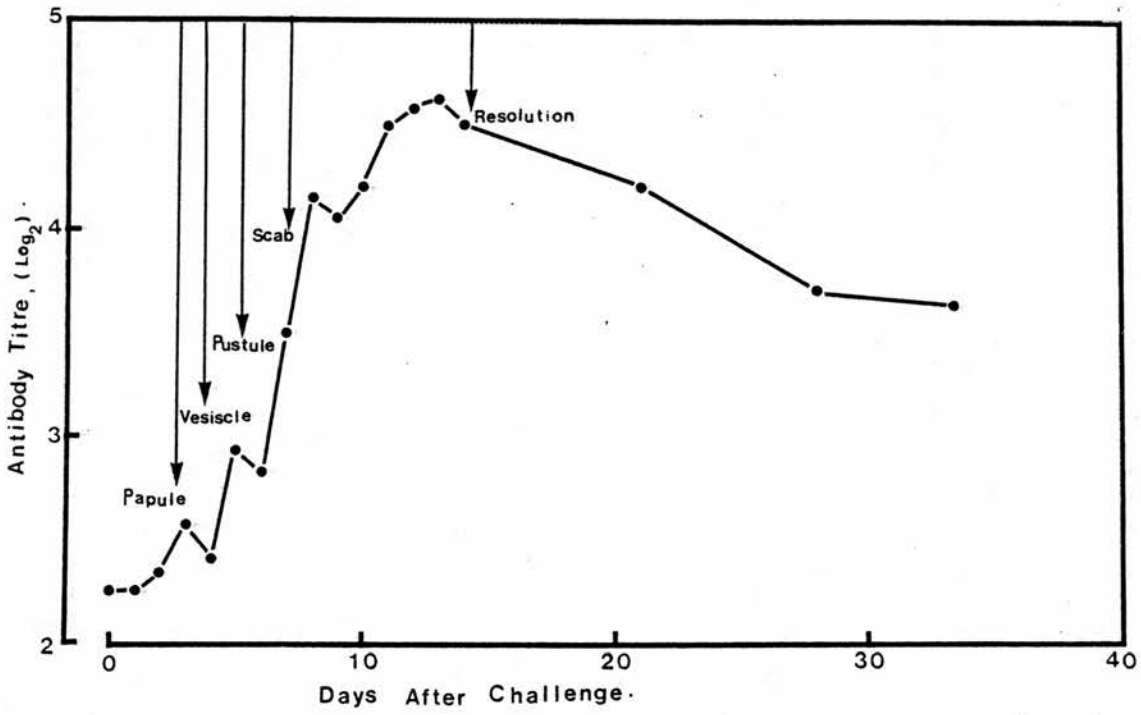


Figure 25 Relationships between orf lesion stages and antibody titres in re-infected sheep.



that the clearance of the virus from the scarified skins of both susceptible and previously infected sheep is associated with the humoral immune response Figures 26 and 27).

Osman (1976) believed that the progressive clinical and pathological changes occurring in sheep experimentally infected or re-infected with orf virus were a result of ~~the~~ active virus multiplication. He tentatively suggested that the presence of antibodies in previously infected sheep were enhancing ~~the~~ virus multiplication and causing the accelerated reaction to re-infection largely because Kapikian and his colleagues (1969), Kim and his colleagues(1969), Chanock and his colleagues (1970) and Smith and his co-workers (1975) had noted this phenomenon in infants and calves infected with respiratory syncytial virus. His attempts, however, to induce an accelerated reaction by injecting susceptible sheep with hyperimmune serum intravenously prior to infecting them with orf virus by scarification failed.

The concept that CMI may be a critical determinant of host resistance to poxvirus infections was first suggested by Pirquet (1907) who speculated that hypersensitivity contributed to lesion of primary local vaccinia virus infection. Later, McKinnon and Defries (1931) concluded that delayed hypersensitivity was the dominant factor in revaccination reactions to smallpox. The suggestions were not widely accepted. However, after the work of Broom (1947) who showed that accelerated re-

Plate 26 Superimposition of the daily means of antibody titres and the growth curve of orf virus in infected susceptible sheep as determined by Osman (1976).

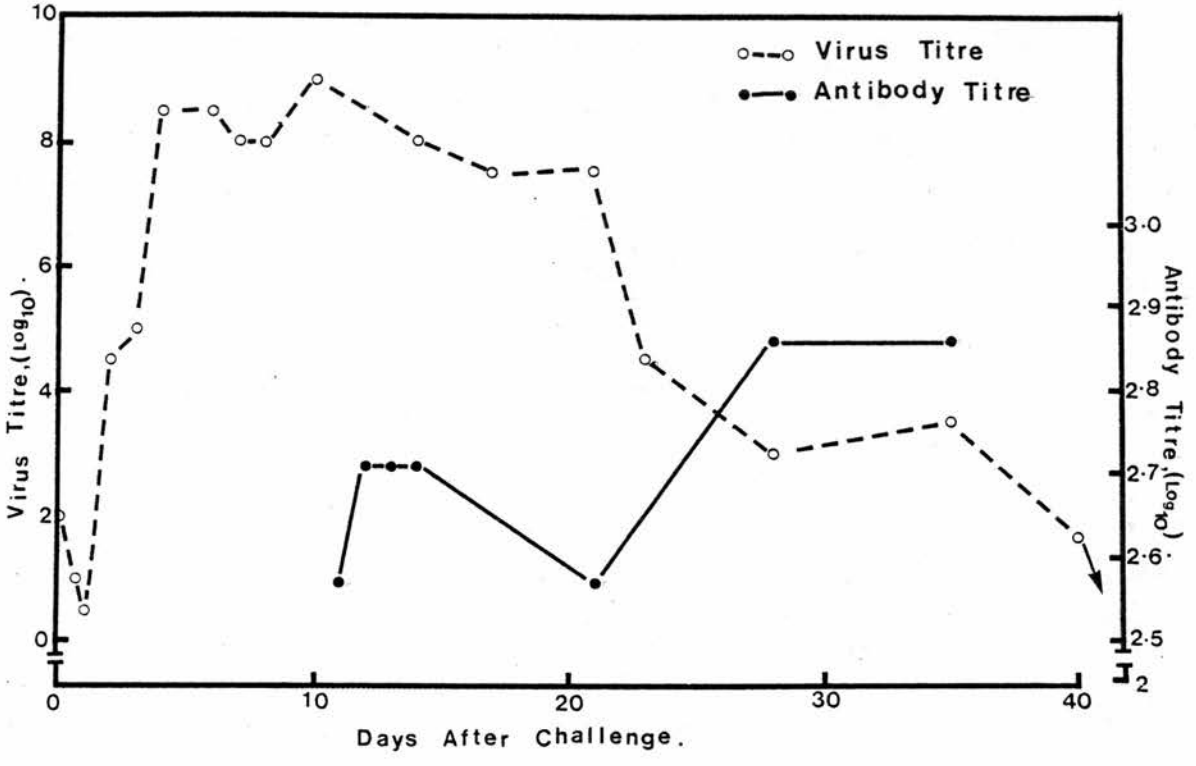
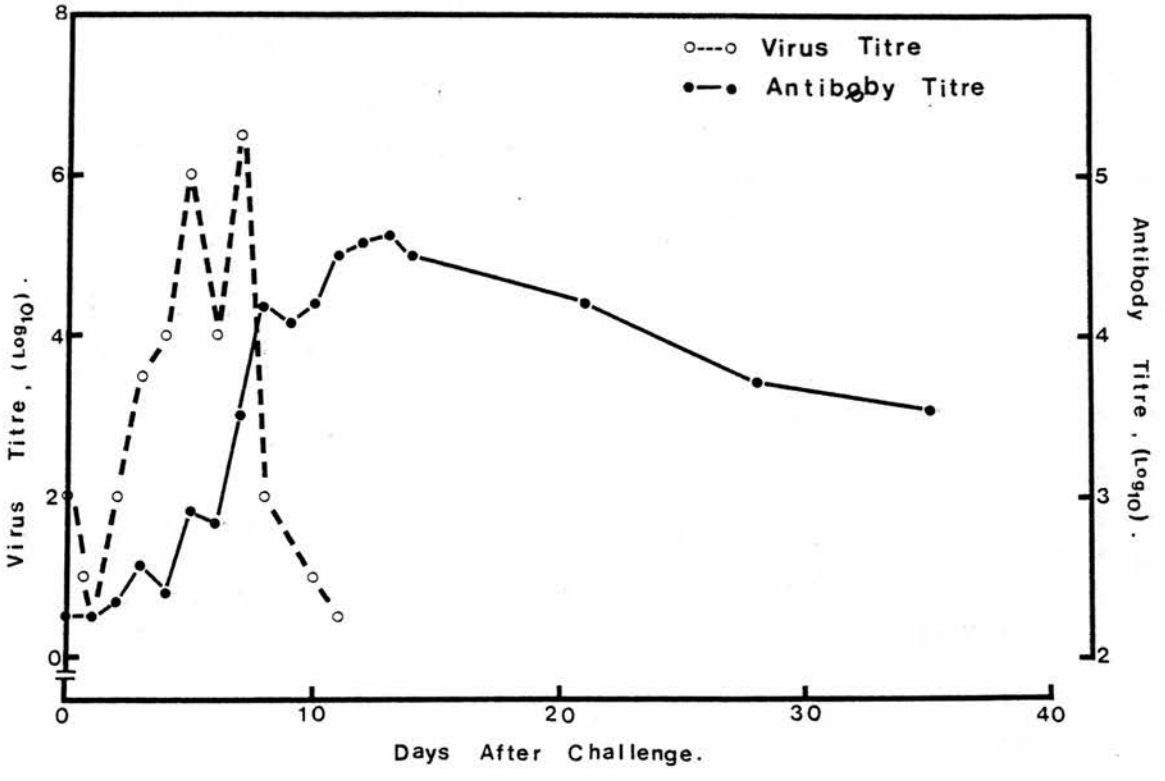


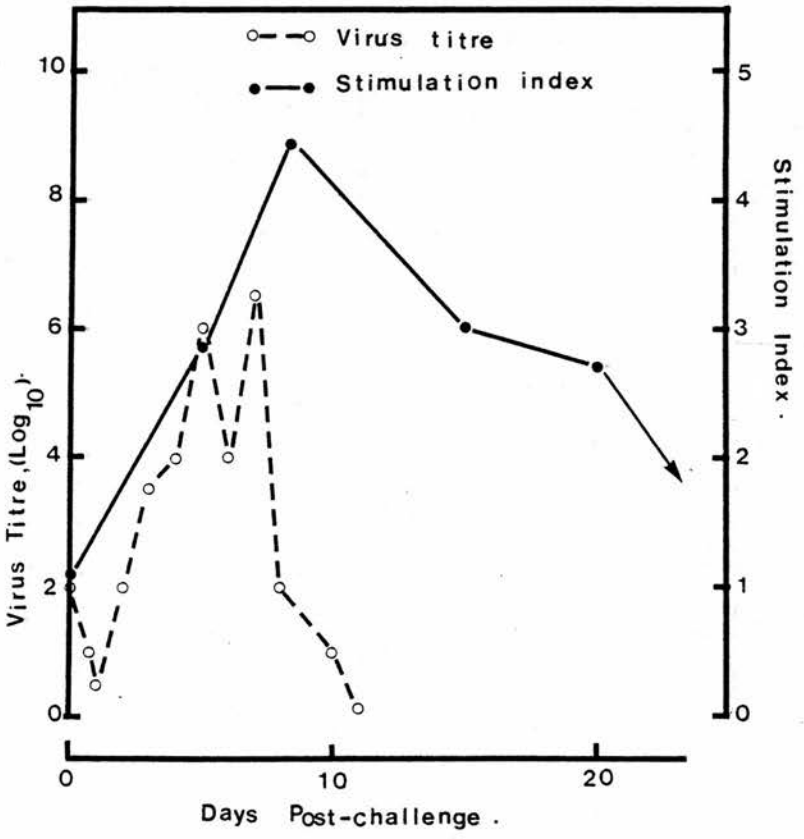
Figure 27 Superimposition of the daily means of the antibody titres and the growth curve of orf virus in previously infected sheep as determined by Osman (1976).



vaccination reactions to vaccinia virus could occur in man in the absence of demonstrable antibody and could be elicited by heated non-infectious virus, and further work by Pincus and Flick (1963) who observed that anti-mononuclear serum inhibited the development of the skin lesions to vaccinia virus though the virus replicated and antibodies were formed, many medical immunologists believed that CMI contributed to primary vaccination and was mainly responsible for the accelerated reactions after re-vaccination to smallpox. Accelerated reactions were, therefore, termed allergic reactions (Allison 1967). The facts that the accelerated reactions had similar pathogenesis as the primary lesion (Pincus and Flick 1963) and that re-vaccination was accompanied with virus multiplication (Kaplan and Morton 1975) have been ignored.

With regard to orf, Osman (1976) failed to incriminate CMI involvement in the one attempt he carried out of transferring lymphoid cells from recovered sheep to susceptible lambs before being infected with orf virus. In the current study, in contrast, I found that injecting susceptible lambs with lymphoid cells from recovering sheep induced an accelerated response. Furthermore, an examination of the relationship between the CMI responses and the growth curves of orf virus in accelerated reactions determined by Osman (1976) revealed that the highest stimulation index was obtained when the virus titre was expected to drop abruptly (Figure 28). Similarly,

Figure 28 Superimposition of the daily means of the 31 values and the growth curve of orf virus in previously infected sheep as determined by Osman (1976).



the abrupt clearance of the virus occurred when the migratory indices were approaching 50 percent (Figure 29). Therefore, it seems not unreasonable to suggest that CMI responses in sheep re-infected with orf virus play a role in the accelerated clinical reactions and virus clearance. However, the comparison between the growth curves of the virus in primary reactions and CMI responses were not so clear-cut; there was an indication that the migratory indices were declining at the same time as the virus titres were waning (Figure 30). More work is required to establish the CMI responses in primary reactions to orf virus.

Observations in man and animals have shown that some pathogenic viruses can suppress the cellular and humoral immune responses in the host. Penhale and Pow (1970) for example, found that rinderpest virus suppressed the humoral immune response of rabbits to chicken erythrocytes; and Mellman and Wetton (1963) found that the attenuated measles virus vaccine suppressed the CMI response of man to tuberculin. Hence, the immunologic dysfunction caused by the viruses are reflected by the changes in the physiologic and immunologic functions of lymphocytes and macrophages since it is the interaction between the antigen and these cells or their products which generate immunity. However, in my study of orf immunology, I did not observe impairment of lymphocyte or macrophage function. Lymphocytes from infected sheep were stimulated with orf antigen <u>in vitro</u> resulting

Figure 29 Superimposition of the daily means of the migratory indices and the growth curve of orf virus in previously infected sheep as determined by Osman (1976).

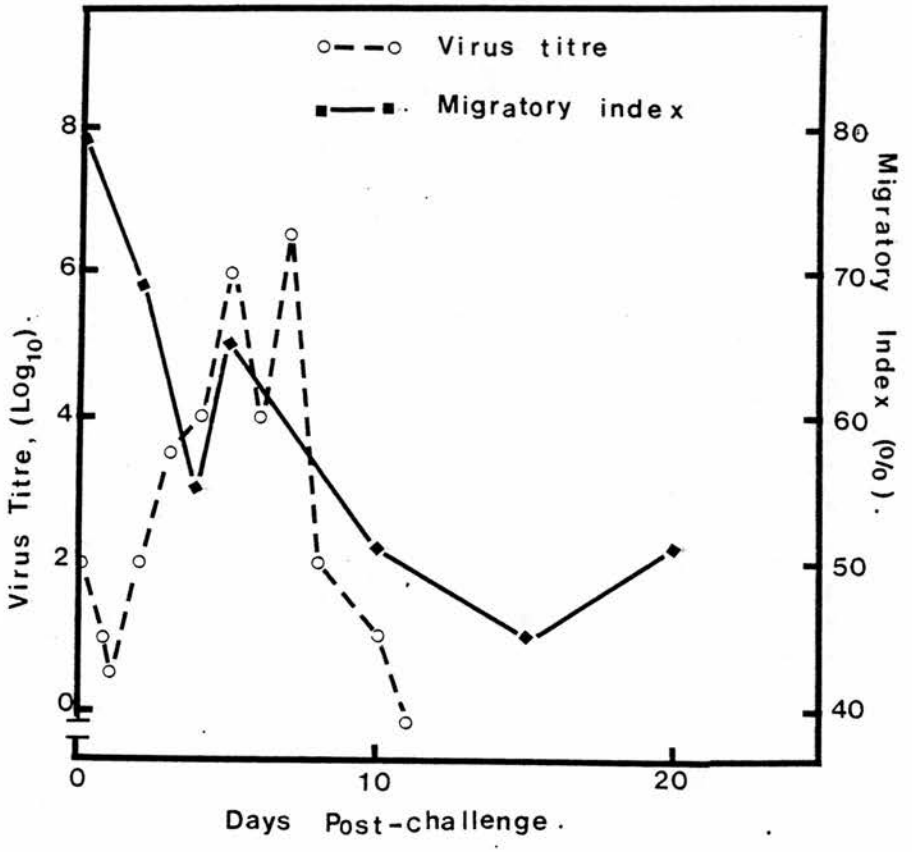
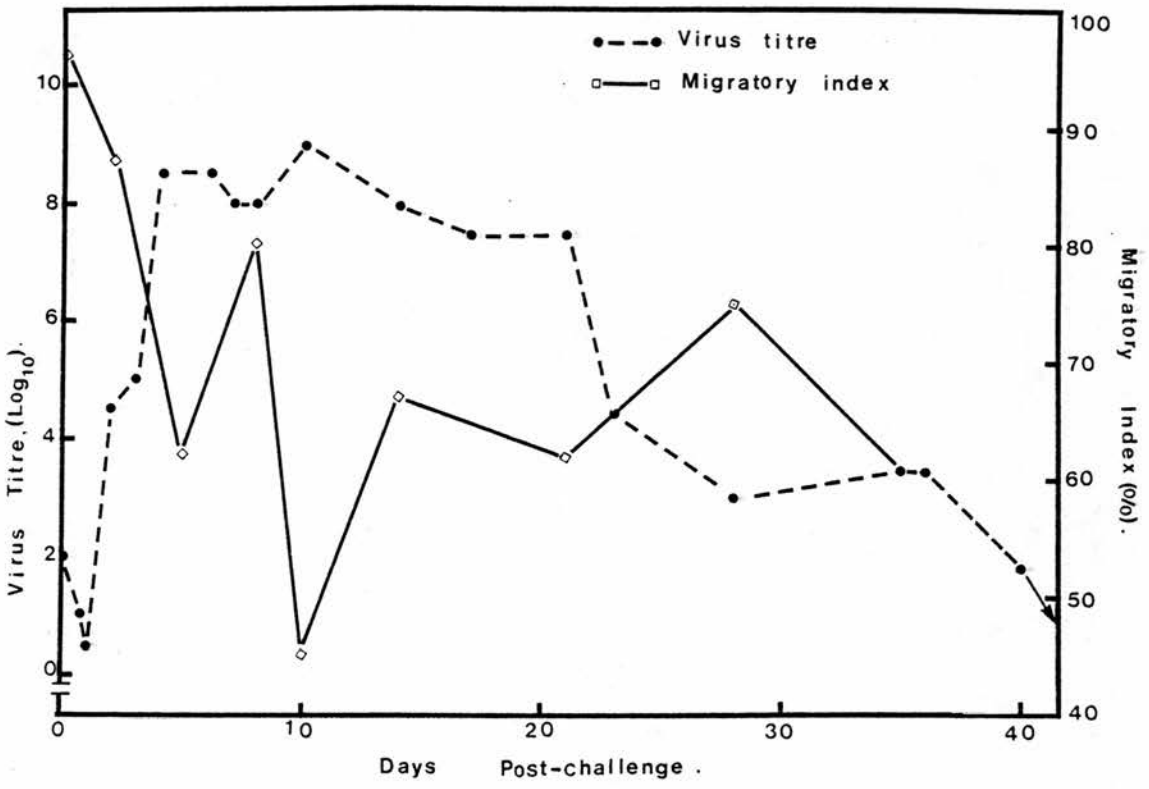


Figure 30 Superimposition of the daily means of Migratory indices and the growth curve of orf virus in infected susceptible sheep as determined by Osman (1976).



in blastogenesis and production MIF and LIF as early as the fourth day after challenge. In addition, antibody production was induced in vivo because orf antibodies were detected in the sera of infected and recovered sheep.

In the attempts to explore the effects of immunosuppression on orf infections I found that the duration of the lesions was longer in the treated sheep than in the untreated sheep irrespective of whether the sheep was susceptible or re-infected. The prolonged course of lesion in the susceptible sheep was mirrored by slower onset of detectable antibodies and the titres attained were slightly lower than those of untreated susceptible sheep. Similarly, the longer course of the lesion in the treated previously infected sheep compared to the untreated previously infected sheep was reflected in the poor and delayed anamnestic antibody response, depression of the levels of serum proteins and IgG1.

There was no evidence of the CMI responses being suppressed in the immunosuppressed susceptible sheep. However, my experiments are open to the criticism that I did not investigate CMI in immunosuppressed previously infected sheep. It would be interesting to monitor the growth and clearance of the virus in treated susceptible and previously infected sheep in relation to the humoral and CMI responses.

The speculation that local skin immunity rather than systemic immune response was operating in orf infections has been made (Anon. 1978) but as yet has not been proven. One could always argue, however, that because antibodies in the skin are believed to come from the blood by transduction (Heremans 1968) there is systemic as well as local involvement. Nevertheless, the concept of local immunity is an attractive proposition and could explain why sheep are refractory to re-infection. Schmidt (1967) found that only 83 percent of the re-infected sheep reacted to the virus and Osman (1976) found that only 94.7 percent of the re-infected sheep had accelerated reactions whilst 5.3 percent were refractory. Fenner and White (1970) had also noticed this non-reacting phenomenon in subjects re-vaccinated against smallpox at the previous site and had found that antibody levels in these refractory individuals did not change so concluded that the booster was not successful. In my study however, all the 38 previously infected sheep reacted to re-infection with an accelerated reaction. Moreover, if local immunity was the operative mechanism how does one explain the accelerated reactions observed in re-infected sheep at different sites since every new site inoculated will be expected to have a primary reaction.

In conclusion, I venture to postulate that both humoral immune and CMI responses are implicated in virus clearance with the humoral immune response being perhaps the more critical of the two.

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APPENDIX II

Buffer Preparations

Barbitone buffer:

Diethyl barbitone 0.331g

Sodium barbitone 1.848g

DDW .....120 ml

pH 8.6, ionic strength 0.075

Hank's balanced salt solution: HBSS is supplied by Wellcome Co. as a sterile 10x concentrate without sodium carbonate. The composition of the concentrate is:-

NaCl	80g/l
KCl	4g/l
KH_2PO_4	0.6g/l
$CaCl_2$	1.4g/l
$MgSO_4$	1.0g/l
$MgCl_2$	1.0g/l
Na_2HPO_4	0.48g/l
Glucose	10 g/l

A single strength of the solution is prepared by diluting the 100ml of the concentrate with 900 ml of DDW and adding 8ml of 4.4 percent sodium bicarbonate.

To prepare HBSS-H, 0.1ml of 5,000 units/ml heparin solution is added to 100ml of HBSS.

Lymphocyte Buffer

Solution A

Anhydrous D. glucose ..... 1g/l.

$CaCl_2$ $2H_20$ .....0.0074 g/l.

$MgCl_2$ .....0.1992 g/l.

KCl .....0.4026 g/l.

Tris hydroxyaminomethone .....17.565 g/l.

Solution B

NaCl ..... 8.19g/l.

Mix one volume of solution A with nine volumes of solution B and sterilize by filtering.

Phosphate Buffer

KH_2PO_4 ..... 20.41g/l.

Na_2HPO_4 ..... 21.30g/l.

Add 28 ml of KH_2PO_4 to 100 ml of Na_2HPO_4 and adjust pH 7.2.

Phosphate Buffered Saline

Purchased from Oxoid Co. in form of tablets containing:-

Sodium Carbonate ..... 8g/l.

Potassium Chloride .....0.2g/l.

Disodium hydrogen phosphate .....1.15g/l.

Potassium dihydrogen phosphate .....0.2g/l.

10 tablets are dissolved in 1 litre of DDW and autoclaved for 10 minutes at 10lb per sq in (115°C).

Sodium Bicarbonate 4.4%

A sterile 4.4% W/V solution of sodium bicarbonate with phenol red indicator is made in DDW. The solution is buffered to pH 7.0 with bubbling in carbon dioxide gas.

Tris-buffered Saline

Tris	12.114g
NaCl	11.69 g
DDW	1 l

pH adjusted to 8.00 by 1N HCl.

Media Preparation

Growth medium for lamb testes and calf testes cell cultures was prepared as follows:-

Eagle's Medium in Earle's salts	10 mls.
New born calf serum	10 mls.
Tryptose broth	10 mls.
Glutamine	200mMoles.
Penicillin	100 units.
Streptomycin	500 ug.
Sodium Bicarbonate (4.4%)	2.5 mls.

Made up to 100 ml by sterile deionized water.

Maintenance medium: was made up as above except the new born calf serum was reduced to 5 ml and sodium carbonate increased to 3 mls.

Growth medium for Vero cell cultures: was prepared as follows:-

TC 199	10mls
Foetal calf serum	10mls
Penicillin	1000 i.u.
Streptomycin	500 ug.

made up to 100mls by sterile deionized water.

Maintenance medium: for vero cells was made in the same way except foetal calf serum added was reduced to 5%.

Tryptose Phosphate Broth: this was commercially purchased from Oxoid Co. in a powder form consisting of:-

Tryptose	20g
Dextrose	2g
NaCl	5g
Na_2HPO_4	2.5g

29.5g of the mixture is dissolved in a one litre of DDW. and then the solution is sterilized by autoclaving for 15 minutes at 15lb per sq in (121°C).

Stains

0.3% Nigrosin Dye prepared as follows:-

Nigrosine	0.3g
PBS	100ml

Ponceau-S dye Solution - used for staining 'phoroslide'

Ponceau-S dye	0.9g
Trichloroacetic acid	13.4g

Sulfosalicylic acid 13.4g

DDW ..... 1 l.

Other Solutions

Alserver Solution: The solution is prepared as follows:-

Sodium Citrate 8g

Dextrose 20.5g

NaCl 4.2g

Citric acid 0.55g

DDW ..... 1 l.

Sterilized by autoclaving 10 p.s.i. for 10 minutes and stored at 4°C.

Cleaning Solution - The solution used for cleaning phoroslide after staining consisted of:-

Glacial acetic acid 70ml

Ethyl acetate 30ml

5% Acetic Acid Solution: made up of:-

Glacial acetic acid 50ml

DDW ..... 950ml.

APPENDIX TABLE 1

Total Serum Protein Contents* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	790	712	362	852	875	836	838	Mean	s.d.
0	75	64	65	65	82	78	77	76	75	86	81	86	82	86	61	54	74.6	9.9
1	78	99	60	64	85	80	78	78	71	78	83	83	67	74	59	52	74.3	11.7
2	84	101	92	70	87	78	78	80	80	74	85	87	N.D.	N.D.	N.D.	N.D.	83.0	8.3
3	64	62	69	65	92	83	104	77	N.D.	N.D.	N.D.	N.D.	74	72	61	52	73.0	14.0
4	N.D.	N.D.	N.D.	N.D.	77	73	85	77	75	80	87	84	N.D.	N.D.	N.D.	N.D.	80.0	5.0
5	74	73	70	77	98	77	87	82	N.D.	N.D.	N.D.	N.D.	71	69	70	65	76.0	9.1
6	73	62	64	68	88	87	87	85	74	79	83	78	N.D.	N.D.	N.D.	N.D.	77.0	9.2
7	82	61	66	62	89	79	83	83	N.D.	N.D.	N.D.	N.D.	53	80	69	64	73.0	11.4
8	75	71	62	62	95	87	94	80	72	74	81	87	N.D.	N.D.	N.D.	N.D.	78.0	11.0
9	70	71	74	72	116	111	92	89	71	81	87	84	72	80	66	62	81.0	15.0
10	62	73	60	63	92	105	95	83	66	78	90	83	71	87	66	62	77.0	14.0
11	62	66	73	81	91	92	102	110	70	80	87	84	69	80	67	64	79.9	14.0
12	87	86	94	82	86	83	91	81	N.D.	N.D.	N.D.	N.D.	72	76	62	64	79.5	9.2
13	86	82	87	87	84	98	81	81	72	80	91	82	69	80	68	66	80.0	7.3
14	87	76	70	79	90	82	85	82	70	78	81	81	72	81	69	69	78.3	6.6
21	93	78	70	89	70	73	74	71	74	76	78	88	76	79	66	63	76.1	8.2
28	93	85	85	82	70	66	74	64	76	76	76	72	69	83	67	68	75.0	8.3
35	70	67	85	83	73	67	69	75	64	78	78	87	70	81	68	68	74.0	7.2

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 2

Albumin Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	790	712	362	852	875	836	838	Mean	s.d.
0	38.00	34.80	44.30	39.10	35.20	36.80	36.40	36.50	26.90	38.60	35.40	41.00	37.40	28.40	37.80	31.30	36.10	4.36
1	47.00	49.70	32.20	38.70	35.60	32.70	36.80	37.60	27.10	34.50	40.00	38.20	29.50	23.00	35.30	31.10	35.60	6.73
2	48.00	30.50	35.00	39.10	36.50	37.60	40.00	33.50	43.00	33.10	35.50	43.60	N.D.	N.D.	N.D.	N.D.	37.95	5.04
3	30.90	39.90	37.80	43.20	39.40	38.30	39.40	31.60	N.D.	N.D.	N.D.	N.D.	33.40	24.50	32.30	26.90	34.80	5.74
4	N.D.	N.D.	N.D.	N.D.	36.20	34.90	41.50	38.50	38.10	37.50	39.20	45.60	N.D.	N.D.	N.D.	N.D.	38.05	1.97
5	30.40	46.60	43.10	43.40	42.10	35.40	41.10	36.00	N.D.	N.D.	N.D.	N.D.	30.00	25.40	32.20	31.20	36.41	6.68
6	31.70	40.50	39.50	41.20	43.20	41.10	42.90	30.50	23.60	41.10	35.60	41.60	N.D.	N.D.	N.D.	N.D.	37.71	6.10
7	34.80	37.40	41.90	40.60	40.00	33.20	38.30	35.00	N.D.	N.D.	N.D.	N.D.	17.00	30.30	30.50	37.60	34.70	6.71
8	47.20	39.30	37.00	38.20	40.90	35.00	44.10	35.90	25.30	37.80	30.90	46.10	N.D.	N.D.	N.D.	N.D.	38.10	6.20
9	43.40	38.90	45.70	47.50	45.30	43.40	40.70	40.90	22.80	29.90	39 10	40.00	27.40	29.50	30.90	38.60	37.70	7.36
10	29.30	39.70	36.50	38.10	40.70	47.30	46.60	44.60	23.90	37.60	39.70	45.60	24.70	24.40	26.60	32.60	36.12	8.23
11	31.10	35.50	47.10	45.60	37.30	37.90	41.60	49.50	30.50	27.90	41.70	52.30	25.60	19.40	28.90	33.10	36.60	9.26
12	42.90	46.50	47.30	54.80	33.60	38.00	38.20	43.00	N.D.	N.D.	N.D.	N.D.	27.40	31.30	28.00	35.70	38.90	8.32
13	43.90	43.40	50.80	54.30	36.10	37.30	37.40	42.20	22.40	31.30	42.50	44.30	30.50	32.70	32.60	37.50	38.70	8.04
14	43.80	42.40	45.10	44.80	34.00	36.90	36.40	36.90	28.70	39.20	34.60	43.60	31.00	28.40	32.60	36.00	37.20	5.56
21	45.60	47.00	49.80	44.80	23.80	27.70	25.20	35.70	31.20	42.30	33.40	33.00	34.00	34.80	32.60	35.90	36.05	7.80
28	44.70	42.50	45.70	45.90	30.80	26.30	28.90	30.30	33.30	37.40	38.90	39.70	46.40	35.50	36.30	42.80	37.80	6.56
35	36.40	36.30	46.60	45.70	34.20	26.90	27.40	36.00	26.40	35.70	32.60	D	40.00	35.50	35.00	40.00	35.65	5.99

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 3

Alpha$_1$-globulin Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	790	712	362	852	875	836	838	Mean	s.d.
0	6.00	1.90	3.90	4.60	6.60	4.70	3.90	4.60	3.70	5.10	4.00	3.40	3.20	4.30	1.80	1.10	3.90	1.4
1	3.10	4.00	3.20	1.80	5.10	4.80	6.30	4.70	3.60	3.90	2.50	4.20	1.30	5.20	1.80	1.60	3.60	1.5
2	7.60	8.10	2.80	3.70	6.10	3.90	2.40	5.60	0.80	3.70	4.20	4.40	N.D.	N.D.	N.D.	N.D.	4.40	2.1
3	2.60	4.40	2.60	4.80	5.50	5.80	8.30	5.40	N.D.	N.D.	N.D.	N.D.	2.20	2.90	2.40	2.60	4.10	1.9
4	N.D.	N.D.	N.D.	N.D.	4.60	5.10	5.90	3.90	2.20	4.00	2.60	3.40	N.D.	N.D.	N.D.	N.D.	4.00	1.2
5	3.70	3.60	1.50	2.80	6.90	5.40	3.50	5.70	N.D.	N.D.	N.D.	N.D.	2.10	2.10	4.20	3.90	3.80	1.6
6	3.60	2.50	1.40	3.90	3.50	3.50	3.50	7.60	4.40	2.40	3.30	3.10	N.D.	N.D.	N.D.	N.D.	3.60	1.5
7	4.90	3.00	2.50	4.70	2.70	1.60	4.20	5.80	N.D.	N.D.	N.D.	N.D.	0.00	4.00	3.50	3.20	3.34	1.6
8	0.70	2.10	3.10	2.50	5.70	3.50	4.70	4.00	3.60	3.00	5.70	4.30	N.D.	N.D.	N.D.	N.D.	3.60	1.6
9	1.40	3.50	5.70	3.00	8.10	5.60	5.50	4.40	5.70	6.50	4.30	3.30	5.00	3.20	3.50	3.10	4.50	1.7
10	1.90	3.70	5.40	3.10	4.60	4.20	2.90	2.60	3.30	3.10	3.60	3.40	4.90	5.00	4.70	3.70	3.80	1.0
11	1.20	4.00	5.10	3.20	3.60	6.50	4.10	2.20	3.50	4.00	2.60	2.60	4.20	5.60	4.70	3.20	3.80	1.3
12	3.50	5.20	5.70	3.80	5.20	4.10	7.30	2.40	N.D.	N.D.	N.D.	N.D.	4.30	2.30	3.70	2.50	4.20	1.5
13	3.40	4.90	6.10	3.50	5.90	3.10	4.10	2.40	6.50	4.00	3.60	3.30	3.50	3.20	4.10	2.00	4.00	1.3
14	3.50	3.60	9.50	5.60	6.30	3.30	3.40	4.10	4.20	1.60	4.00	3.20	4.30	4.10	4.20	2.80	4.20	1.8
21	3.70	2.40	3.60	4.90	4.20	4.40	5.90	2.90	3.00	1.50	2.30	3.50	4.50	4.00	3.30	3.80	3.60	1.1
28	4.70	2.50	4.10	5.90	2.80	2.60	3.70	3.90	3.00	3.10	1.50	2.90	6.90	4.10	4.00	4.80	3.80	1.4
35	2.80	4.00	5.00	5.90	3.60	3.40	5.50	4.50	3.20	3.90	3.10	3.50	5.00	3.90	4.10	4.90	4.10	0.9

s.d. – standard deviation

N.D. – Not Done *– g/l

APPENDIX TABLE 4

Alpha$_2$-globulin Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	790	712	362	852	875	836	838	Mean	s.d.
0	6.00	5.20	9.80	3.30	9.80	10.20	8.50	9.10	9.00	10.30	10.50	11.10	8.10	9.50	5.50	7.50	8.30	2.2
1	5.50	14.90	9.70	6.00	11.00	10.40	9.40	7.80	7.80	10.20	10.80	10.80	7.40	7.40	5.90	7.30	8.90	2.5
2	7.60	11.10	11.20	8.30	8.70	9.40	9.40	10.40	8.00	11.00	11.00	10.50	N.D.	N.D.	N.D.	N.D.	9.70	1.3
3	5.20	5.60	10.40	1.40	11.00	10.80	10.40	11.60	N.D.	N.D.	N.D.	N.D.	7.4	7.20	6.70	7.80	8.00	3.0
4	N.D.	N.D.	N.D.	N.D.	8.50	9.50	8.50	9.20	8.20	9.60	10.50	9.30	N.D.	N.D.	N.D.	N.D.	9.20	0.7
5	3.70	7.30	12.30	6.30	10.80	9.20	11.40	12.30	N.D.	N.D.	N.D.	N.D.	7.90	4.80	9.10	9.10	8.70	2.8
6	10.20	5.60	10.90	5.80	8.80	9.60	11.40	12.70	8.80	10.30	9.10	7.80	N.D.	N.D.	N.D.	N.D.	9.20	2.1
7	11.50	5.50	8.60	6.70	8.90	10.30	10.00	9.20	N.D.	N.D.	N.D.	N.D.	6.40	10.40	8.30	9.60	8.80	1.8
8	3.00	7.90	6.80	7.40	10.50	10.50	11.30	8.80	8.00	8.90	8.90	10.40	N.D.	N.D.	N.D.	N.D.	8.50	2.2
9	3.50	5.70	11.40	6.70	13.90	13.40	11.10	9.80	7.10	8.90	10.40	8.30	5.00	8.80	8.60	8.10	8.80	2.9
10	2.50	8.80	7.60	7.10	10.20	11.60	10.50	9.10	7.30	8.60	11.70	8.50	7.10	9.20	10.60	12.30	8.90	2.4
11	4.40	6.60	10.60	9.60	10.90	11.10	10.20	12.10	7.70	8.80	10.40	8.60	7.60	9.60	8.70	10.80	9.20	2.0
12	10.50	8.60	9.00	9.50	9.50	9.10	10.00	8.90	N.D.	N.D.	N.D.	N.D.	8.70	6.90	8.10	10.20	9.10	1.0
13	6.00	8.20	9.60	11.40	8.40	8.50	10.60	8.10	9.40	9.60	10.90	9.90	6.20	6.40	8.80	10.50	9.10	1.5
14	12.30	8.30	8.70	8.40	11.60	9.80	8.50	9.80	7.70	7.60	9.70	8.90	7.90	10.60	9.00	9.00	9.20	1.3
21	13.00	7.10	12.40	8.40	9.80	10.20	10.40	8.60	7.40	7.60	10.10	11.40	6.80	8.70	8.60	8.80	9.30	1.8
28	10.20	7.60	11.40	11.00	8.40	8.60	11.90	7.10	7.60	9.20	9.20	7.20	7.60	8.30	7.40	8.10	8.80	1.5
35	9.80	7.40	10.00	9.30	8.70	9.40	9.60	9.80	7.10	9.30	10.90	8.70	7.70	8.00	7.40	8.80	8.90	1.1

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 5

Beta-globulin Levels* of Sheep Re-infected with Orf Virus – Group I

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	790	712	362	852	875	836	838	Mean	s.d.
0	7.50	2.60	6.50	9.10	6.60	10.20	5.40	7.30	8.20	9.40	8.10	8.60	4.90	5.20	3.70	3.20	6.66	2.3
1	7.80	8.90	12.90	3.60	6.80	12.80	6.30	7.10	7.80	8.60	7.50	9.10	4.00	5.20	4.10	3.60	7.26	2.9
2	9.20	7.10	14.00	3.70	8.60	10.20	7.80	5.60	8.00	6.60	6.80	8.70	N.D.	N.D.	N.D.	N.D.	8.02	2.5
3	8.40	4.40	8.50	5.50	10.10	10.00	10.40	4.60	N.D.	N.D.	N.D.	N.D.	4.50	2.90	3.70	4.10	6.40	2.8
4	N.D.	N.D.	N.D.	N.D.	7.70	9.50	7.60	5.40	8.20	9.60	7.00	8.40	N.D.	N.D.	N.D.	N.D.	7.90	
5	14.10	5.10	10.00	4.20	9.80	13.10	8.80	4.90	N.D.	N.D.	N.D.	N.D.	7.10	4.80	4.20	5.20	7.60	3.5
6	6.60	5.00	4.80	4.50	6.20	12.30	9.60	6.80	8.80	5.50	9.90	8.60	N.D	N.D.	N.D.	N.D.	7.40	2.4
7	8.20	6.70	4.70	4.70	4.40	10.30	6.70	6.70	N.D.	N.D.	N.D.	N.D.	6.40	5.60	4.90	3.20	6.00	1.9
8	6.70	7.10	5.50	4.90	10.50	11.40	7.50	7.20	7.10	7.30	7.00	9.20	N.D.	N.D.	N.D.	N.D.	7.60	1.9
9	6.30	7.10	7.10	5.20	10.50	14.50	6.40	7.20	8.00	7.30	8.10	7.00	4.30	8.80	4.60	2.50	7.20	2.7
10	10.60	5.10	6.00	4.40	10.50	9.50	9.20	6.20	7.30	4.70	9.90	8.50	5.70	4.20	4.70	3.70	6.90	2.4
11	11.80	5.90	7.40	5.70	12.70	11.10	6.10	5.00	9.10	8.80	7.80	9.40	5.50	2.40	4.70	5.10	7.40	2.9
12	11.40	6.90	8.50	5.70	7.70	10.70	7.30	6.60	6.50	8.00	7.20	5.70	5.00	3.10	3.10	3.20	6.70	2.4
13	7.40	6.60	7.90	5.00	7.60	10.10	8.10	4.10	8.40	6.30	8.10	8.10	4.90	3.20	3.40	3.30	6.40	2.1
14	13.10	6.00	5.60	4.70	8.10	13.10	6.80	4.90	8.40	6.30	8.10	8.10	3.60	4.10	2.80	4.90	6.80	3.0
21	9.30	6.30	7.10	6.30	9.80	11.60	8.90	4.80	7.40	6.00	8.50	7.00	4.50	3.20	4.00	3.80	6.80	2.4
28	7.40	6.80	8.20	6.80	7.00	9.20	8.20	5.70	9.10	9.60	4.60	6.50	6.90	4.10	4.00	4.10	6.80	1.9
35	5.20	5.40	7.60	5.60	5.10	8.70	6.90	5.20	7.10	8.50	6.20	6.90	5.10	4.00	4.00	3.80	6.00	1.5

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 6

Gammaglobulin Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	712	790	362	852	875	836	838	Mean	s.d.
0	21.0	10.3	8.5	9.1	23.8	16.4	22.5	21.3	23.9	22.5	22.3	22.2	27.6	38.5	12.2	10.4	19.5	8.0
1	14.1	21.9	6.4	9.5	26.3	19.2	19.6	21.2	22.8	22.5	18.8	20.8	22.1	33.4	11.8	8.6	18.7	7.0
2	16.0	22.2	7.0	17.6	26.9	17.2	20.4	21.5	20.7	27.1	19.1	17.4	N.D.	N.D.	N.D.	N.D.	19.4	5.3
3	17.4	12.5	5.9	13.7	25.7	18.3	24.9	23.9	N.D.	N.D.	N.D.	N.D.	26.7	34.6	15.8	10.4	19.2	8.2
4	N.D.	N.D.	N.D.	N.D.	20.0	13.8	18.6	23.9	19.4	27.9	19.9	17.7	N.D.	N.D.	N.D.	N.D.	20.2	4.2
5	22.3	18.1	6.9	12.6	28.4	13.9	22.8	21.3	N.D.	N.D.	N.D.	N.D.	24.3	31.6	20.3	9.1	19.3	7.5
6	20.4	8.7	6.8	9.0	26.5	21.0	21.0	23.7	27.2	24.8	19.8	16.5	N.D.	N.D.	N.D.	N.D.	18.4	7.1
7	22.9	9.1	6.2	10.0	24.0	22.1	23.3	28.3	N.D.	N.D.	N.D.	N.D.	23.4	38.3	22.2	10.2	20.0	9.4
8	19.5	15.0	6.2	8.6	27.6	24.5	27.2	25.5	27.5	27.6	20.7	19.1	N.D.	N.D.	N.D.	N.D.	20.8	7.5
9	17.5	15.6	12.1	10.4	38.3	34.5	25.9	27.6	28.4	26.1	28.3	22.5	30.3	37.5	18.4	10.0	24.0	9.2
10	16.8	16.2	13.9	9.5	31.4	32.6	26.7	24.8	24.6	25.3	24.3	18.6	28.3	41.2	20.0	9.2	22.7	8.6
11	15.6	13.8	14.6	13.2	29.1	26.8	30.5	34.1	25.9	24.3	30.3	20.6	26.3	39.9	20.2	11.5	23.5	8.3
12	22.8	18.9	14.7	18.0	30.1	23.1	27.3	24.4	N.D.	N.D.	N.D.	N.D.	26.7	32.8	19.3	12.1	22.5	6.2
13	21.5	18.8	17.5	15.6	25.2	23.3	21.9	23.5	27.5	26.2	30.4	18.9	24.3	34.3	19.0	12.5	22.5	5.6
14	21.9	15.1	15.8	14.0	29.6	18.8	24.6	23.8	23.8	24.2	21.2	21.0	25.2	34.1	20.8	16.6	21.9	5.3
21	24.2	15.7	17.8	11.2	22.4	18.9	23.7	20.7	25.2	23.3	18.1	22.8	25.7	28.5	18.0	10.7	20.4	5.0
28	25.1	15.2	15.2	12.3	21.0	19.1	21.5	18.0	23.4	22.1	19.1	17.3	20.8	30.6	15.5	8.1	19.0	5.3
35	21.0	13.4	16.1	17.5	21.1	18.8	19.2	20.3	20.6	24.9	20.2	20.0	N.D.	N.D.	N.D.	N.D.	19.4	2.8

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 7

I_gG_1 Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	712	790	362	852	875	836	838	Mean	s.d.
0	18.7	7.8	16.9	8.9	18.7	10.5	14.8	20.8	18.6	11.6	12.1	14.3	21.3	36.0	13.0	8.8	15.8	6.9
1	13.0	12.0	5.1	8.0	17.5	11.1	12.6	22.4	19.3	12.8	11.1	16.6	22.4	32.5	13.2	7.5	15.0	6.8
2	17.2	11.5	10.8	16.1	17.8	10.7	12.2	22.0	19.3	15.0	13.2	13.2	N.D.	N.D.	N.D.	N.D.	15.1	3.6
3	12.9	9.0	10.7	14.4	19.2	11.5	13.7	18.7	N.D.	N.D.	N.D.	N.D.	23.2	32.0	14.2	5.2	15.4	7.1
4	N.D.	N.D.	N.D.	N.D.	17.5	14.4	25.0	17.0	21.2	17.4	14.2	14.2	N.D.	N.D.	N.D.	N.D.	17.6	3.8
5	14.3	10.2	9.0	9.8	12.8	12.8	18.3	21.4	N.D.	N.D.	N.D.	N.D.	23.2	32.0	17.2	6.3	15.6	7.3
6	14.3	17.2	8.0	14.4	13.7	20.8	19.6	21.5	18.6	16.2	13.2	13.2	N.D.	N.D.	N.D.	N.D.	15.9	3.9
7	12.9	11.5	12.7	16.1	15.0	23.8	17.8	21.6	N.D.	N.D.	N.D.	N.D.	23.1	36.0	20.6	6.3	18.1	7.7
8	11.5	17.2	8.7	19.7	15.9	23.8	18.3	22.5	18.6	13.9	15.4	15.4	N.D.	N.D.	N.D.	N.D.	16.8	4.3
9	9.0	18.7	14.8	19.7	19.2	25.4	24.7	27.7	15.8	15.0	14.3	14.3	21.4	34.0	21.6	6.3	18.9	7.0
10	11.5	25.3	12.8	17.9	22.2	26.1	25.0	22.5	12.8	13.9	15.4	15.5	26.5	32.1	18.5	11.3	19.3	6.4
11	10.2	10.3	14.4	23.5	27.2	23.8	29.0	32.5	19.6	18.6	16.6	16.6	28.4	32.1	17.0	10.3	20.6	7.7
12	15.7	11.5	16.1	25.5	22.2	19.2	29.6	34.0	N.D.	N.D.	N.D.	N.D.	24.8	40.0	18.5	8.8	22.2	9.2
13	11.5	11.5	9.9	19.7	20.7	27.1	32.2	36.0	22.6	16.2	16.2	15.5	24.8	40.0	20.0	12.1	21.0	9.0
14	14.3	11.2	12.8	25.5	22.2	29.4	35.7	32.1	22.8	19.9	16.6	16.6	26.6	34.1	22.0	11.3	22.1	8.0
21	18.7	12.9	11.2	25.4	19.2	18.0	33.9	22.5	22.5	16.9	13.2	13.2	26.6	34.0	23.3	7.5	19.9	7.6
28	15.7	13.0	23.5	27.5	20.7	19.7	21.3	18.6	26.8	16.7	15.4	14.3	26.5	34.0	21.5	11.3	20.9	6.1
35	15.7	17.2	17.9	25.3	20.7	18.0	23.9	20.2	16.2	16.2	13.2	14.3	24.0	32.0	20.2	10.0	19.1	5.4

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 8

I_gG_2 Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	712	790	362	852	875	836	838	Mean	s.d.
0	2.2	3.0	9.6	4.2	5.8	2.6	3.4	7.6	9.8	6.0	4.7	5.6	5.6	11.2	6.6	3.5	5.7	2.7
1	2.0	2.9	9.5	3.9	7.5	3.4	5.3	7.7	9.8	5.3	4.7	5.6	5.6	10.8	7.3	3.3	5.6	2.8
2	2.2	2.6	10.4	4.8	8.7	2.6	5.3	7.7	9.8	5.3	5.6	5.6	N.D.	N.D.	N.D.	N.D.	5.9	2.8
3	2.2	2.6	10.0	3.1	8.1	3.4	4.4	8.7	N.D.	N.D.	N.D.	N.D.	6.2	10.4	8.7	4.1	6.0	3.0
4	N.D.	N.D.	N.D.	N.D.	7.8	3.4	5.8	7.4	8.9	6.0	5.6	5.6	N.D.	N.D.	N.D.	N.D.	6.3	1.7
5	2.6	2.6	10.4	3.7	7.8	3.3	5.3	6.9	N.D.	N.D.	N.D.	N.D.	5.0	12.4	8.7	3.5	6.0	3.2
6	2.4	2.6	9.6	4.2	6.9	3.0	5.3	9.4	9.8	6.7	6.5	5.6	N.D.	N.D.	N.D.	N.D.	6.2	2.5
7	2.2	3.0	9.6	4.2	6.9	2.6	5.8	7.0	N.D.	N.D.	N.D.	N.D.	5.0	12.8	7.3	4.1	5.9	3.1
8	1.8	2.6	11.2	3.7	6.9	3.0	4.9	6.3	6.7	6.7	6.5	5.6	N.D.	N.D.	N.D.	N.D.	5.5	2.5
9	2 2	3.4	12.1	3.1	8.1	3.4	4.4	6.8	7.4	6.7	6.5	5.6	5.6	14.6	6.7	4.7	6.2	3.3
10	1.5	3.0	9.6	4.2	11.6	3.0	6.3	7.3	6.7	6.3	6.6	6.4	5.1	14.6	5.9	4.1	6.4	3.3
11	2.2	3.8	12.1	4.2	7.5	3.6	5.3	6.3	6.8	6.6	9.3	7.4	6.2	13.6	5.9	5.3	6.6	3.1
12	2.2	2.6	9.6	5.4	8.1	3.0	5.8	7.3	N.D.	N.D.	N.D.	N.D.	6.3	13.7	6.6	4.7	6.3	3.2
13	2.2	1.8	11.2	4.8	8.5	3.0	5.8	8.4	6.8	6.6	7.4	7.3	5.8	12.8	6.6	3.5	6.5	3.0
14	1.8	1.8	9.6	4.5	10.8	2.5	4.9	8.2	6.9	6.0	6.5	6.4	5.6	11.2	6.6	5.5	6.2	2.8
21	2.3	2.6	10.4	4.2	6.9	2.4	4.6	7.3	6.5	6.1	6.3	8.3	5.6	10.4	6.6	5.3	6.0	2.5
28	2.0	2.5	9.7	5.4	8.5	2.4	3.3	7.8	5.9	6.0	6.4	8.3	6.2	11.2	5.9	5.3	6.05	2.6
35	2.1	2.5	9.7	6.1	6.9	2.4	3.8	7.2	5.4	5.3	6.0	6.5	5.0	10.8	6.0	4.7	5.7	2.3

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 9

I_gM_1 Levels* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	712	790	362	852	875	836	838	Mean	s.d.
0	4.3	3.3	3.0	4.9	4.4	3.4	3.0	3.2	1.4	3.1	5.2	4.1	4.5	4.5	2.7	2.2	3.6	1.0
1	4.5	3.5	3.2	5.0	4.5	5.0	4.0	3.8	1.4	2.6	5.2	4.1	3.4	4.0	4.7	2.7	3.8	1.0
2	5.0	3.8	3.5	5.5	7.8	6.0	3.8	3.5	1.4	3.1	5.2	5.3	N.D.	N.D.	N.D.	N.D.	4.5	1.6
3	4.9	3.8	3.6	4.9	8.4	5.5	3.5	4.0	N.D.	N.D.	N.D.	N.D.	4.2	2.4	5.9	2.7	4.4	1.9
4	N.D.	N.D.	N.D.	N.D.	5.5	7.7	4.9	3.5	1.3	2.6	4.7	5.1	N.D.	N.D.	N.D.	N.D.	4.4	1.9
5	5.5	3.3	3.0	5.5	5.5	5.0	4.8	4.1	N.D.	N.D.	N.D.	N.D.	4.0	2.9	3.7	2.7	4.2	1.1
6	4.9	2.9	2.6	4.4	5.7	5.1	3.5	3.5	1.8	2.6	4.7	6.1	N.D.	N.D.	N.D.	N.D.	4.0	1.4
7	4.3	3.3	2.1	3.9	4.2	5.5	4.0	2.7	N.D.	N.D.	N.D.	N.D.	4.0	2.4	3.7	3.2	3.6	0.9
8	4.9	2.9	2.1	4.4	3.9	4.9	3.2	1.7	0.8	2.6	5.6	3.9	N.D.	N.D.	N.D.	N.D.	3.4	1.4
9	4.3	4.9	2.6	4.9	3.3	3.3	2.7	2.7	0.8	2.6	5.4	4.1	4.5	3.4	4.2	3.7	3.6	1.2
10	4.3	3.8	2.1	4.9	3.3	5.5	4.1	3.3	3.5	2.6	5.6	4.1	5.1	3.4	5.3	3.7	3.9	1.0
11	4.9	3.8	2.6	4.9	3.1	3.3	3.2	2.9	1.0	2.2	5.4	3.9	5.1	4.5	5.3	3.7	3.7	1.2
12	4.9	2.9	2.6	6.0	4.5	5.5	3.7	2.7	N.D.	N.D.	N.D.	N.D.	4.5	4.5	5.3	4.7	4.3	1.1
13	4.9	2.9	2.6	6.0	5.0	5.0	3.8	2.7	1.0	2.6	4.7	3.9	4.9	4.5	4.7	3.7	3.9	1.3
14	4.4	3.0	2.5	6.1	5.2	5.8	4.5	2.7	1.0	2.6	4.7	3.9	4.5	4.7	3.2	3.7	3.9	1.3
21	5.4	3.8	3.5	6.6	3.6	4.5	2.9	2.6	1.5	2.5	4.5	3.5	4.5	3.4	3.2	3.6	3.7	1.2
28	4.3	3.5	3.9	6.6	3.5	3.5	3.3	2.6	1.6	2.2	4.9	3.0	4.5	3.5	4.2	3.2	3.6	1.1
35	4.9	4.3	3.5	5.0	3.5	4.5	2.9	2.7	2.0	2.2	4.7	4.1	4.0	3.4	3.9	2.7	3.6	0.9

s.d. – standard deviation

N.D. – Not Done * – g/l

APPENDIX TABLE 10

Orf Antibody Titres* of Sheep Re-infected with Orf Virus – Group 1

Days Post Challenge	NUMBER OF SHEEP																STATISTICS	
	751	780	749	765	767	779	768	691	740	712	790	362	852	875	836	838	Mean	s.d.
0	3	3	2	3	3	0	3	3	0	0	4	4	2	2	2	2	2.25	1.29
1	3	3	2	3	3	0	3	3	0	0	4	4	2	2	2	2	2.25	1.29
2	3	3	2	3	3	0	3	3	0	0	4	4	N.D.	N.D.	N.D.	N.D.	2.33	1.50
3	4	3	2	3	3	0	3	3	N.D.	N.D.	N.D.	N.D.	2	2	3	3	2.58	1.00
4	N.D.	N.D.	N.D.	N.D.	3	0	3	3	0	0	5	5	N.D.	N.D.	N.D.	N.D.	2.37	2.13
5	4	3	3	3	3	2	3	3	N.D.	N.D.	N.D.	N.D.	2	2	4	3	2.92	0.67
6	4	3	3	3	3	2	3	3	0	0	5	5	N.D.	N.D.	N.D.	N.D.	2.83	1.58
7	4	4	4	3	4	3	4	4	N.D.	N.D.	N.D.	N.D.	2	2	4	4	3.50	0.80
8	5	4	4	4	4	4	5	4	2	2	6	6	N.D.	N.D.	N.D .	N.D.	4.17	1.27
9	5	4	4	4	6	4	5	4	2	2	6	7	2	2	4	4	4.06	1.53
10	5	5	4	4	6	4	5	4	2	2	7	6	2	2	5	4	4.19	1.56
11	5	5	4	5	5	4	5	4	3	3	7	7	3	3	5	4	4.50	1.26
12	5	5	4	5	6	5	6	5	N.D.	N.D.	N.D.	N.D.	3	2	5	4	4.58	1.16
13	5	4	4	5	6	5	6	5	4	3	6	6	3	3	5	4	4.62	1.09
14	5	4	4	5	5	5	6	6	4	3	5	5	3	3	5	4	4.50	0.97
21	4	4	4	5	4	4	5	6	4	2	5	5	3	4	4	4	4.19	0.91
28	4	4	3	4	4	4	4	4	3	2	5	5	2	4	4	3	3.69	0.87
35	4	4	3	4	4	3	4	4	3	2	5	5	2	4	4	3	3.62	0.88

s.d. – standard deviation

N.D. – Not Done * – $\log_2$

APPENDIX TABLE 10a

ORF ANTIBODY TITRES* OF SHEEP WHICH HAD DETECTABLE ANTIBODIES BEFORE RE-INFECTION WITH ORF VIRUS (GROUP I)

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	751	780	765	767	768	691	790	362	Mean	s.d.
0	3	3	3	3	3	3	4	4	3.25	0.46
1	3	3	3	3	3	3	4	4	3.25	0.46
2	3	3	3	3	3	3	4	4	3.25	0.46
3	4	3	3	3	3	3	N.D.	N.D.	3.17	0.41
4	N.D.	N.D.	N.D.	3	3	3	5	5	3.80	1.10
5	4	3	3	3	3	3	N.D.	N.D.		
6	4	3	3	3	3	3	5	5	3.62	0.92
7	4	4	3	4	4	4	N.D.	N.D.	3.83	0.41
8	5	4	4	4	5	4	6	6	4.75	0.89
9	5	4	4	6	5	4	6	7	5.12	1.12
10	5	5	4	6	5	4	7	6	5.25	1.04
11	5	5	5	5	5	5	7	7	5.50	0.93
12	5	5	5	6	6	5	N.D.	N.D.	5.33	0.52
13	5	4	5	6	6	5	6	6	5.38	0.74
14	5	4	5	5	6	6	5	5	5.12	0.64
21	4	4	5	4	5	6	5	5	4.75	0.71
28	4	4	4	4	4	4	5	5	4.25	0.46
35	4	4	4	4	4	4	5	5	4.25	0.46

S.D. = STANDARD DEVIATION.

N.D. = NOT DONE.

* = $\log_2$

APPENDIX TABLE 10b.

ORF ANTIBODY TITRE* OF SHEEP WHICH HAD NO OR ONLY TRACES OF ANTIBODIES BEFORE RE-INFECTION WITH ORF VIRUS (GROUP 1).

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	749	779	740	712	852	875	836	838	Mean	s.d.
0	2	0	0	0	2	2	2	2	1.25	1.03
1	2	0	0	0	2	2	2	2	1.25	1.03
2	2	0	0	0	N.D.	N.D.	N.D.	N.D.	0.50	1.00
3	2	0	N.D.	N.D.	2	2	3	3	2.00	1.10
4	N.D.	0	0	0	N.D.	N.D.	N.D.	N.D.	0.00	0.00
5	3	2	N.D.	N.D.	2	2	4	3	2.67	0.82
6	3	2	0	0	N.D.	N.D.	N.D.	N.D.	1.25	1.50
7	4	3	N.D.	N.D.	2	2	4	4	3.17	0.98
8	4	4	2	2	N.D.	N.D.	N.D.	N.D.	3.00	1.15
9	4	4	2	2	2	2	5	4	3.12	1.25
10	4	4	2	2	2	2	5	4	3.12	1.25
11	4	4	3	3	3	3	5	4	3.62	0.74
12	4	5	N.D.	N.D.	3	2	5	4	3.83	1.17
13	4	5	4	3	3	3	5	4	3.88	0.83
14	4	5	4	3	3	3	5	4	3.88	0.83
21	4	4	4	2	3	4	4	4	3.62	0.74
28	3	4	3	2	2	4	4	3	3.12	0.83
35	3	3	3	2	2	4	4	3	3.00	0.76

s.d. = standard deviation

N.D. = Not Done.

* $\log_2$

APPENDIX TABLE 11.

TOTAL SERUM PROTEIN CONTENTS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	69	67	65	70	75	69.2	3.77
1	72	66	67	68	69	68.4	2.30
2	66	61	67	66	68	65.0	2.64
3	68	66	63	68	74	67.8	4.02
4	62	63	62	69	67	64.6	3.21
5	63	65	62	63	65	63.6	1.34
6	71	71	63	65	72	68.4	4.10
7	71	63	65	69	74	68.4	4.45
8	69	65	64	67	69	66.8	2.28
9	70	67	63	65	68	66.6	2.70
10	71	69	65	61	65	66.2	3.90
11	69	63	65	63	72	66.4	3.97
12	68	66	61	63	67	65.0	2.92
13	67	67	68	68	64	66.8	1.64
14	65	66	64	69	74	67.6	4.04
21	68	66	64	68	74	68.0	3.74
28	68	68	65	68	76	69.0	4.12
35	71	65	64	66	71	67.4	3.36

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 12.

ALBUMIN LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP OF GROUP 2

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	43.0	33.5	22.5	32.2	29.4	32.12	7.42
1	44.2	32.2	34.8	31.8	29.1	34.4	5.83
2	43.8	28.3	33.3	30.2	28.0	32.7	6.54
3	31.6	32.3	31.4	30.8	29.4	31.1	1.09
4	33.0	31.4	32.0	31.9	27.5	31.2	2.13
5	31.4	29.1	29.2	22.0	22.6	26.9	4.27
6	39.5	35.3	34.0	32.6	30.2	34.3	3.46
7	39.9	33.0	34.6	34.0	30.9	34.5	3.34
8	37.4	32.3	39.2	26.9	22.9	31.7	6.88
9	41.6	34.0	33.6	27.5	24.6	32.3	6.58
10	36.7	34.7	34.3	28.9	20.1	30.9	6.71
11	39.4	27.0	34.0	29.6	24.3	30.9	5.96
12	35.1	33.6	36.3	23.9	28.7	31.5	5.15
13	31.2	33.9	35.8	34.1	32.4	33.5	1.75
14	33.2	35.2	36.5	40.2	28.0	34.6	4.50
21	32.7	34.1	34.6	35.1	29.4	33.2	2.29
28	36.5	38.2	35.6	30.4	31.4	34.4	3.36
35	37.4	43.8	34.6	35.9	29.9	36.12	4.67

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 13.

ALPHA$_1$-GLOBULIN LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP GROUP 2.

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	2.10	4.0	2.6	3.5	3.8	3.20	0.82
1	1.40	2.6	2.0	2.6	2.8	2.28	0.58
2	3.0	1.2	2.6	1.3	2.7	2.16	0.84
3	2.0	3.1	3.7	2.7	2.9	2.88	0.62
4	2.5	3.1	2.5	3.5	4.0	3.12	0.65
5	6.3	3.9	3.1	4.4	2.6	4.06	1.43
6	2.1	2.8	2.5	2.6	4.3	2.86	0.84
7	2.1	3.2	2.6	1.4	4.4	2.74	1.14
8	2.8	3.5	2.6	3.0	3.9	3.16	0.53
9	2.1	4.4	2.6	1.8	3.3	2.84	1.04
10	2.0	2.6	2.6	3.1	3.9	2.84	0.71
11	2.1	3.35	2.6	3.8	4.3	3.23	0.89
12	2.7	4.0	5.4	3.1	4.0	3.84	1.04
13	3.3	2.0	2.7	4.8	2.6	3.08	1.07
14	3.3	2.7	2.6	3.5	3.7	3.16	0.49
21	2.0	2.7	2.6	2.7	4.4	2.88	0.90
28	2.0	5.4	1.9	2.7	3.1	2.22	0.68
35	2.8	0.65	1.3	1.3	4.3	2.07	1.47

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 14.

ALPHA$_2$ GLOBULIN LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP

GROUP 2

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	6.90	8.0	9.0	11.9	9.0	9.0	1.85
1	7.20	9.2	10.0	10.1	8.3	8.97	1.21
2	8.6	11.0	9.0	8.5	6.8	8.78	1.50
3	8.5	7.5	9.2	10.0	9.3	8.90	0.95
4	8.1	6.9	9.2	9.7	8.7	8.52	1.08
5	10.0	8.4	9.3	10.0	7.7	9.08	1.01
6	9.9	8.5	9.4	8.5	8.6	8.98	0.64
7	9.2	7.0	9.1	9.0	8.1	8.48	0.94
8	9.9	8.3	7.8	7.3	7.8	8.22	1.00
9	9.0	7.6	8.4	8.8	7.2	8.20	0.77
10	8.8	9.0	6.5	10.0	6.5	8.16	1.58
11	8.0	8.8	10.4	9.4	8.7	9.06	0.90
12	9.1	7.2	10.9	9.4	8.0	8.92	1.41
13	9.5	8.7	10.1	12.3	6.4	9.40	2.14
14	7.8	9.3	9.0	7.6	7.4	8.22	0.87
21	9.5	8.0	9.0	9.5	7.4	8.68	0.94
28	7.4	8.9	9.7	11.5	8.4	9.18	1.54
35	9.2	6.5	9.0	9.3	8.5	8.50	1.16

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 15.

BETA-GLOBULIN LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	4.2	4.0	2.6	4.9	6.0	4.34	1.25
1	5.1	3.3	3.3	5.4	5.5	4.53	1.11
2	6.6	1.8	3.8	6.6	4.1	4.58	2.03
3	5.3	3.1	4.3	2.7	3.6	3.80	1.04
4	8.1	3.8	4.9	3.5	4.0	4.86	1.89
5	5.0	3.9	4.3	8.8	5.8	5.57	1.94
6	4.9	4.2	3.1	3.9	5.7	4.37	0.98
7	5.7	3.8	3.9	4.2	5.9	4.70	1.02
8	4.2	3.5	3.2	4.3	4.6	3.96	0.59
9	4.9	3.5	3.9	4.4	5.8	4.50	0.90
10	6.3	3.5	3.2	2.5	5.2	4.14	1.56
11	5.5	3.8	3.9	3.8	5.1	4.42	0.81
12	5.4	2.6	6.6	3.1	5.4	4.62	1.70
13	7.4	3.3	3.4	4.9	2.6	4.33	1.91
14	5.9	2.6	2.6	4.1	4.4	3.93	1.37
21	6.1	2.0	3.8	6.1	5.4	4.68	1.77
28	6.8	4.1	3.2	5.4	5.4	4.98	1.38
35	5.6	2.6	3.8	5.3	5.5	4.56	1.32

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 16

GAMMAGLOBULIN LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	13.2	17.4	15.5	17.5	27.1	18.14	5.31
1	14.5	18.4	16.7	17.6	23.6	18.2	3.37
2	15.9	16.5	15.4	15.7	23.2	17.3	3.30
3	15.1	16.2	16.0	16.1	25.1	17.7	4.16
4	12.4	17.6	16.0	17.4	24.1	17.5	4.24
5	13.0	19.4	16.2	17.6	24.5	18.1	4.26
6	14.1	19.8	14.8	15.0	23.0	17.3	3.89
7	16.4	16.5	13.8	13.9	24.3	17.0	4.29
8	15.0	18.7	15.0	15.0	30.7	18.1	6.80
9	14.5	17.5	14.0	16.5	25.5	17.6	4.64
10	15.5	18.7	13.6	17.1	26.7	18.3	5.05
11	13.8	18.9	14.4	15.7	24.6	17.5	4.44
12	14.9	19.7	14.5	17.5	18.1	16.9	2.20
13	16.7	18.8	15.5	16.5	17.9	17.09	1.28
14	15.0	15.9	16.5	15.6	23.6	17.3	3.50
21	17.7	16.6	14.7	14.6	22.1	17.1	3.07
28	14.9	15.7	14.2	17.6	23.0	17.1	3.54
35	15.5	17.5	15.4	16.6	19.9	17.2	2.14

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 17.

IgG_1 LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	276	833	Mean	s.d.
0	11.10	12.70	13.5	12.70	12.70	12.55	0.86
1	12.70	11.10	12.70	12.70	13.5	12.55	0.86
2	11.90	11.90	11.90	13.5	13.5	12.55	0.86
3	11.10	11.90	12.70	11.90	11.9	11.92	0.55
4	11.90	12.70	14.3	11.90	11.9	12.55	1.02
5	12.70	11.90	11.90	13.5	14.3	12.86	1.02
6	11.10	11.90	13.5	12.70	13.5	12.55	1.02
7	11.90	11.10	12.76	12.70	13.5	12.40	0.90
8	11.10	11.10	11.92	13.5	13.5	12.24	1.19
9	11.90	11.90	12.76	12.70	14.3	12.71	0.96
10	12.70	10.30	11.92	12.70	14.3	12.39	1.42
11	12.70	11.10	14.27	11.90	14.3	12.86	1.40
12	11.90	11.90	14.27	11.10	13.5	12.55	1.29
13	11.10	11.10	12.70	11.92	13.5	12.08	1.02
14	11.10	11.90	12.70	12.70	13.5	12.39	0.89
21	10.30	11.90	12.70	11.10	13.5	11.92	1.24
28	10.30	11.90	12.70	11.10	13.5	11.92	1.24
35	11.90	12.70	12.70	11.10	13.5	12.39	0.89

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 18.

IgG_2 LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	5.94	4.45	4.45	4.82	4.08	4.75	0.72
1	5.17	4.45	4.45	4.45	4.08	4.52	0.40
2	5.57	4.45	4.45	4.64	4.45	4.71	0.49
3	5.57	4.45	4.08	4.64	4.45	4.64	0.56
4	5.56	4.45	4.45	4.45	4.45	4.67	0.50
5	5.57	4.45	4.45	4.45	4.82	4.75	0.49
6	5.94	4.54	4.82	4.82	4.82	4.99	0.55
7	5.56	4.54	4.45	4.82	4.45	4.76	0.47
8	5.56	4.45	4.45	4.45	4.82	4.75	0.48
9	5.57	4.45	4.54	4.64	4.45	4.71	0.48
10	5.56	4.45	4.54	4.82	4.45	4.76	0.47
11	5.56	4.45	4.45	5.94	4.45	4.97	0.72
12	5.94	4.82	4.82	4.82	4.45	4.97	0.56
13	5.94	4.82	4.45	4.82	4.82	4.97	0.56
14	4.82	4.45	4.82	4.82	4.45	4.67	0.20
21	4.82	4.45	4.82	4.82	4.45	4.67	0.20
28	4.82	4.45	4.54	4.82	4.08	4.54	0.31
35	5.94	4.45	4.45	4.82	4.82	4.90	0.61

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 19.

IgM LEVELS* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2.

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	3.16	3.16	3.16	3.46	4.07	3.40	0.395
1	3.16	3.16	3.46	3.86	4.37	3.40	0.58
2	2.56	2.86	3.77	3.16	4.07	3.28	0.63
3	2.56	2.86	3.77	3.16	4.37	3.34	0.73
4	2.86	3.16	3.76	3.46	4.37	3.52	0.58
5	2.86	2.26	3.46	3.16	4.97	3.34	1.01
6	2.86	2.56	3.47	3.16	4.67	3.34	0.82
7	2.86	2.86	3.16	3.16	5.27	3.46	1.02
8	2.86	3.16	3.47	3.77	4.97	3.65	0.81
9	2.86	3.16	3.77	3.16	4.97	3.58	0.84
10	3.16	3.16	3.76	3.47	5.27	3.76	0.88
11	2.86	3.46	3.76	3.16	4.97	3.64	0.81
12	2.86	3.16	4.07	3.16	4.96	3.64	0.87
13	3.16	3.46	3.76	3.46	4.67	3.70	0.58
14	2.86	3.46	4.07	3.16	4.96	3.70	0.83
21	3.16	3.16	4.37	3.16	4.07	3.58	0.59
28	3.16	2.86	4.37	3.46	4.37	3.64	0.70
35	3.16	2.56	3.46	3.64	4.07	3.38	0.56

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 20.

ORF ANTIBODY TITRES* OF UNCHALLENGED PREVIOUSLY INFECTED SHEEP - GROUP 2

Days Post-Challenge	NUMBER OF SHEEP					STATISTICS	
	22	38	39	376	833	Mean	s.d.
0	2	2	2	0	2	1.6	0.89
1	2	2	2	0	2	1.6	0.89
2	2	2	2	2	3	2.2	0.45
3	2	2	2	2	2	2.0	0.00
4	2	2	2	2	2	2.0	0.00
5	2	2	2	2	2	2.0	0.00
6	2	2	2	2	2	2.0	0.00
7	2	2	2	2	2	2.0	0.00
8	2	2	2	2	2	2.0	0.00
9	2	2	2	2	2	2.0	0.00
10	2	2	2	3	2	2.2	0.45
11	2	2	2	2	3	2.2	0.45
12	2	2	2	2	2	2.0	0.00
13	2	2	2	2	2	2.0	0.00
14	2	2	2	2	2	2.0	0.00
21	2	2	2	2	2	2.0	0.00
28	2	2	2	2	2	2.0	0.00
35	2	2	2	2	2	2.0	0.00

s.d. = standard deviation.

* = $\log_2$.

APPENDIX TABLE 21.

TOTAL SERUM PROTEIN CONTENTS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	67	68	67	73	71	81	71	71.1	4.5
1	69	67	70	71	69	73	65	69.3	2.4
3	58	62	65	73	70	69	66	66.2	4.7
5	68	69	73	69	65	75	68	69.2	3.2
7	64	64	65	67	66	67	67	66.7	3.2
8	71	81	71	67	66	74	69	90.7	4.9
9	70	78	70	68	65	69	63	68.7	4.5
10	70	77	74	70	64	69	66	70.4	4.2
11	69	73	73	70	63	77	68	70.7	4.2
12	71	68	74	70	64	73	74	69.6	4.3
13	71	72	75	77	71	69	69	72.0	3.0
14	68	73	72	69	68	68	65	69.0	2.7
21	74	74	81	69	68	71	66	71.9	5.0
28	66	66	74	77	69	72	68	70.3	4.2
35	68	69	64	74	67	69	66	68.1	3.1

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 22.

ALBUMIN LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	34.80	32.80	28.00	34.40	36.00	24.20	29.20	30.90	4.19
1	37.00	32.80	28.10	37.80	32.30	21.90	30.00	31.80	5.19
3	26.20	26.80	24.20	35.90	35.70	27.80	20.40	27.70	5.46
5	32.10	30.20	24.20	35.30	32.70	31.70	19.10	28.90	5.40
6	29.30	27.70	25.50	28.70	31.70	22.80	27.50	27.85	2.72
7	28.20	30.30	28.50	27.00	30.10	22.80	29.60	27.50	2.87
8	35.50	35.00	26.60	30.10	32.70	22.00	29.50	20.54	4.82
9	33.80	39.20	27.40	32.20	32.10	22.00	32.50	30.40	5.61
10	34.90	35.50	26.80	28.00	30.60	25.50	24.30	29.30	4.12
11	34.70	34.40	27.30	29.40	32.10	25.50	29.70	30.62	3.25
12	32.50	32.10	22.40	32.40	31.40	23.40	28.10	28.90	4.37
13	34.30	35.10	23.00	29.10	28.60	21.50	23.30	27.83	5.50
14	33.30	39.30	20.60	29.80	33.80	23.20	27.60	30.51	5.38
21	34.50	39.70	25.80	31.70	38.10	23.30	32.10	32.20	6.01
28	31.00	31.90	25.80	31.70	38.10	27.30	32.50	31.19	3.97
35	28.70	32.60	25.00	28.10	36.20	26.40	31.30	29.76	3.87

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 23.

LEVELS*
ALPHA$_1$-GLOBULIN/OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS -

GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	3.30	4.10	2.70	2.20	3.50	1.60	4.30	3.16	0.93
1	3.40	3.30	2.10	1.40	4.10	2.90	3.90	3.16	0.99
3	4.10	3.70	3.30	4.40	3.50	3.50	3.90	3.71	0.39
5	4.10	4.10	5.10	2.80	2.60	3.80	3.40	3.91	0.99
6	4.30	3.00	3.30	3.30	3.30	3.40	4.00	3.62	0.53
7	4.50	3.20	3.60	2.70	4.00	3.70	4.10	3.99	1.02
8	5.70	4.90	3.50	2.10	3.30	2.90	3.80	4.02	1.38
9	2.80	3.10	3.70	2.10	3.80	2.80	3.20	3.50	1.33
10	3.50	3.90	1.50	3.50	3.10	3.40	4.10	3.60	1.19
11	3.50	4.40	3.00	4.20	3.2	3.10	4.50	3.79	0.65
12	5.00	4.10	2.20	3.90	3.60	2.20	3.40	3.49	1.01
13	5.00	3.60	3.60	3.50	4.80	2.70	3.90	3.87	0.79
14	4.10	4.40	3.20	2.10	3.40	3.50	3.90	3.51	0.75
21	4.40	3.70	3.70	3.10	3.50	2.90	2.10	3.34	0.73
28	3.30	3.30	3.70	3.10	3.50	2.90	2.10	3.13	0.52
35	4.10	3.50	3.20	3.70	2.70	3.50	2.00	3.24	0.70

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 24.

ALPHA$_2$ - GLOBULIN LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	9.40	12.30	8.00	8.80	8.50	9.70	8.50	9.30	1.33
1	9.60	12.70	7.00	7.80	8.90	8.70	7.80	8.69	1.86
3	12.20	11.20	6.50	9.50	9.80	9.70	8.60	9.60	1.69
5	13.00	13.00	7.30	9.00	9.20	8.30	10.20	9.76	2.17
6	11.00	11.50	5.90	9.30	11.20	10.00	8.70	9.56	1.83
7	11.50	11.60	6.40	10.80	9.90	8.80	7.60	9.40	1.87
8	12.80	15.50	4.90	12.30	8.50	8.30	7.50	9.89	3.40
9	12.70	12.50	6.70	10.50	9.00	8.90	7.30	9.90	2.28
10	10.50	12.40	8.00	9.10	7.50	9.30	10.10	9.72	1.59
11	11.80	11.70	7.40	8.40	8.30	9.50	10.40	9.69	1.60
12	12.00	11.60	8.20	9.30	10.00	9.70	8.90	9.96	1.39
13	12.10	12.20	5.80	7.60	9.50	7.50	9.70	9.20	2.41
14	10.90	10.90	8.10	9.00	8.10	9.90	9.20	9.44	1.18
21	14.00	10.30	8.10	10.00	7.60	10.00	8.90	9.84	2.10
28	10.50	11.30	7.60	10.00	7.60	10.00	8.90	9.41	1.43
35	12.30	12.50	7.70	9.60	9.40	9.70	9.30	10.10	1.73

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 25.

BETA-GLOBULIN LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	4.00	5.50	4.00	4.40	6.40	7.30	5.00	5.11	1.21
1	4.80	4.70	4.20	3.60	6.20	4.40	3.90	4.50	0.79
3	5.20	6.20	5.20	5.10	4.90	4.90	5.90	5.42	0.52
5	5.50	4.80	5.10	5.50	4.60	6.00	3.40	4.86	0.86
6	4.30	4.20	4.60	4.70	4.00	5.40	3.40	4.37	0.58
7	5.80	3.20	4.30	5.40	4.60	3.70	4.10	4.47	0.85
8	7.10	7.30	4.20	3.40	3.90	4.10	3.80	4.89	1.53
9	4.90	6.30	3.70	3.50	3.80	3.40	3.30	4.16	1.02
10	5.60	6.20	4.40	5.60	5.00	3.10	5.20	4.75	1.20
11	4.90	5.80	4.40	4.90	4.50	3.70	5.50	4.52	1.04
12	4.90	4.10	5.20	6.20	7.80	3.30	6.20	5.39	1.50
13	5.00	5.70	5.00	5.50	7.50	3.40	5.80	5.41	1.22
14	4.8	2.90	4.80	2.80	5.40	4.20	4.60	4.21	1.00
21	7.40	3.70	4.80	4.60	4.90	2.90	5.50	4.83	1.42
28	4.00	3.30	4.40	4.60	4.90	2.90	5.50	4.23	0.91
35	4.80	4.20	4.50	6.70	5.40	4.60	4.00	4.89	0.92

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 26.

GAMMAGLOBULIN LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	15.4	13.7	26.4	23.5	16.3	27.9	24.2	21.0	5.7
1	13.7	13.4	28.5	20.7	17.2	25.0	19.6	19.7	5.6
3	10.5	14.4	26.2	18.3	16.1	23.6	27.0	19.4	6.3
5	13.7	16.5	31.6	16.6	16.4	25.6	23.5	20.6	6.5
6	12.2	13.9	N.D.	N.D.	N.D.	25.6	32.1	20.9	9.5
7	14.1	16.1	26.2	20.7	15.9	34.6	23.4	21.6	7.2
8	17.0	18.7	28.2	20.9	17.2	N.D.	N.D.	20.4	4.6
9	16.2	17.2	30.8	20.5	16.4	31.6	18.2	21.6	6.7
10	15.3	19.3	32.6	21.7	15.4	32.3	19.9	22.4	7.3
11	14.6	16.8	31.9	23.8	16.3	30.5	24.3	22.3	8.0
12	16.3	16.4	31.7	23.1	16.0	33.6	23.7	22.0	7.4
13	15.0	15.1	36.6	25.5	18.5	32.6	21.9	22.6	8.5
14	14.9	15.3	34.6	23.6	17.7	31.4	22.0	22.0	7.7
21	13.2	16.2	32.0	24.9	16.9	33.9	20.4	22.5	8.0
28	17.1	16.6	28.8	27.8	15.2	28.7	19.8	22.0	6.2
35	18.4	16.6	30.0	25.7	13.4	25.0	20.0	21.3	5.8

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 27

IgG_1 LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	5.1	4.0	12.0	8.2	14.0	21.0	17.4	11.7	6.3
1	6.2	5.6	12.8	8.2	13.9	21.0	15.7	11.9	5.6
3	4.6	6.7	12.8	9.0	13.9	22.7	16.6	12.3	6.2
5	5.7	7.3	12.8	8.2	13.5	17.2	15.7	11.5	4.4
6	6.2	8.4	N.D.	N.D.	N.D.	20.1	13.9	12.2	6.2
7	8.0	7.3	12.8	9.7	13.5	20.1	15.7	12.4	4.5
8	11.2	6.7	13.5	10.5	13.4	N.D.	N.D.	11.1	2.8
9	10.0	7.3	13.5	9.7	15.7	21.0	15.6	13.3	4.6
10	12.4	7.1	12.8	9.7	15.7	25.4	15.6	14.1	5.8
11	7.8	7.3	16.6	9.7	15.7	25.2	15.7	14.0	6.3
12	6.7	7.8	11.3	8.2	14.9	22.8	16.6	12.6	5.8
13	7.8	10.4	11.3	9.00	15.7	20.1	15.6	12.8	4.4
14	8.9	8.7	12.0	11.3	15.7	19.2	17.4	13.3	4.2
21	8.4	9.0	12.8	11.3	15.6	21.0	15.7	13.4	4.4
28	9.5	9.0	12.8	11.3	13.0	17.4	15.7	12.7	3.1
35	6.7	8.4	6.7	12.8	13.9	18.3	15.7	11.8	4.6

s.d. = standard deviation

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 28.

IgG_2 LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	2.5	1.4	6.1	3.4	4.1	7.5	5.1	4.4	2.0
1	2.5	1.4	6.6	2.0	4.1	7.8	5.1	4.3	2.2
3	1.9	2.5	7.8	3.9	4.4	8.2	5.1	4.6	2.1
5	3.0	2.5	4.8	3.0	4.4	7.8	5.4	4.6	1.7
6	3.0	3.0	N.D.	N.D.	N.D.	7.8	5.4	4.8	2.3
7	2.5	3.0	6.6	3.4	4.4	7.8	5.4	4.6	1.8
8	2.5	2.5	6.1	3.9	4.4	N.D.	N.D.	4.0	1.4
9	2.5	4.0	7.5	4.3	4.7	7.8	5.8	5.1	1.8
10	1.9	3.6	6.6	4.3	4.7	8.2	5.8	5.0	1.9
11	3.0	3.5	5.7	4.3	4.7	9.6	5.4	5.0	2.1
12	3.5	3.5	6.6	3.9	4.7	8.2	5.8	4.8	1.9
13	3.5	3.5	6.6	4.3	4.4	8.5	6.1	4.9	2.0
14	3.5	3.5	6.6	3.0	4.4	8.9	6.5	5.2	2.2
21	3.0	4.0	5.7	5.2	4.7	7.8	6.1	5.1	1.5
28	3.0	3.5	5.7	3.8	4.4	8.7	5.8	5.0	2.0
35	3.0	3.5	6.1	4.3	4.7	8.7	5.8	4.6	1.1

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 29

IgM LEVELS* OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3

Days Post Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	2.6	3.3	3.1	1.6	4.2	4.0	4.0	3.3	0.93
1	2.6	2.8	2.7	1.6	3.7	4.5	4.2	3.2	1.0
3	1.6	1.6	2.4	2.0	3.9	4.0	4.2	2.8	1.2
5	2.6	2.3	2.2	1.8	4.5	4.2	4.2	3.1	1.1
6	1.9	2.6	N.D.	N.D.	N.D.	4.7	4.0	3.3	1.3
7	2.3	2.1	1.8	2.0	4.5	4.2	4.2	3.0	1.2
8	2.1	2.8	1.8	1.8	4.2	N.D.	N.D.	2.6	0.9
9	1.6	2.8	1.3	1.8	5.6	4.0	4.2	3.0	1.5
10	2.6	2.9	1.8	2.4	5.0	4.0	3.7	3.2	1.0
11	2.1	2.8	1.6	1.8	4.2	4.0	4.0	2.9	1.1
12	2.3	2.6	1.8	2.2	4.6	4.0	4.2	3.2	1.0
13	2.6	1.4	2.7	1.4	4.5	4.0	4.2	3.0	1.2
14	2.8	2.3	1.8	2.7	4.2	4.0	4.6	3.2	1.1
21	2.3	2.3	2.0	1.8	5.0	4.0	4.0	3.1	1.3
28	2.1	2.3	2.7	2.0	4.6	4.0	4.0	3.2	1.2
35	2.1	2.3	2.7	2.0	4.6	4.2	4.0	3.1	1.1

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 30

ORF ANTIBODY TITRES*OF SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS

GROUP 3

Days Post-Challenge	NUMBER OF SHEEP							STATISTICS	
	39	40	52	65	67	63	68	Mean	s.d.
0	2	2	2	0	0	0	0	0.86	1.07
1	2	2	2	0	0	0	0	0.86	1.07
3	2	2	2	0	0	0	0	0.86	1.07
5	2	2	2	0	0	0	0	0.86	1.07
6	2	2	N.D.	N.D.	N.D.	0	2	1.50	1.00
7	2	2	2	0	0	0	2	1.14	1.07
8	2	2	2	2	0	N.D.	N.D.	1.60	0.89
9	2	2	2	3	0	0	2	1.57	1.13
10	3	2	2	3	0	0	2	1.71	1.25
11	3	3	3	2	2	2	3	2.57	0.53
12	3	3	3	3	2	2	3	2.71	0.48
13	3	3	3	3	2	2	3	2.71	0.48
14	4	3	3	2	2	2	3	2.71	0.76
21	4	3	2	3	2	2	2	2.57	0.79
28	4	4	2	4	2	2	2	2.86	1.07
35	3	3	2	4	3	3	2	2.86	0.69

s.d. = standard deviation

N.D. = Not Done.

* = $\log_2$.

APPENDIX TABLE 31.

MIGRATION INDICES OF LEUCOCYTES FROM UNTREATED SUSCEPTIBLE SHEEP INFECTED WITH ORF VIRUS - GROUP 3.

Days post-Challenge	NUMBER OF SHEEP 39	40	52	65	67	63	68	Mean	s.d.
- 48 hrs.	93.4	89.7	100.7	94.0	80.5	90.0	10.20	94	7.0
0	100.0	90.3	101.0	-	-	-	-	98	5.0
2	87.9	122.0	120.9	55.1	44.1	56.6	56.2	87	41.0
5	-	-	73.3	13.2	50.5	91.1	53.4	62	29.0
8	128.0	80.8	90.5	60.0	45.2	75.6	94.0	80	25.0
10	-	-	-	17.7	72.3	-	-	45	39.0
14	70.0	75.8	75.1	76.4	68.4	55.0	48.0	67	11.0
21	83.3	55.0	86.9	61.0	92.0	22.0	36.0	62	27.0
28	56.8	79.0	101.0	61.0	76.0	-	-	75	17.0
35	83.6	74.3	-	59.5	64.0	47.0	37.0	61	17.0

APPENDIX TABLE 32.

MIGRATION INDICES OF LEUCOCYTES FROM SHEEP RE-INFECTED WITH ORF VIRUS - GROUP 4.

Days Post-Challenge	833	836	780	842	10	12	38	39	67	83	32	51	69	126	Mean	s.d.
0	62	79	84	97	102	112	63	49	77	51	86	55	79	110	79	21
2	76	76	64	60	-	-	-	-	-	-	-	-	-	-	69	8
4	66	68	36	50	-	-	-	-	-	-	-	-	-	-	55	15
5	61	-	-	-	79	105	-	44	45	52	51	62	78	42	65	19
10	48	42	51	52	55	66	68	47	62	44	37	52	45	41	51	9
15	29	39	-	-	54	62	43	48	64	33	44	34	42	49	45	11
20	-	-	-	-	53	48	47	64	61	57	47	44	43	45	51	7
42	-	-	-	-	50	45	61	58	59	65	47	44	43	46	52	8
63	-	-	-	-	59	35	58	50	75	45	50	74	69	85	60	16

S.D. = Standard Deviation

- = Not Done.

APPENDIX TABLE 33.

MIGRATION INDICES OF LEUCOCYTES FROM PREVIOUSLY INFECTED SHEEP NOT CHALLENGED - GROUP 5.

Days Post-Challenge	779	740	775	877	82	69	84	51	32	34	Mean	s.d.
0	100	80	75	53	43	59	84	74	52	44	66.4	19.0
2	97	71	88	-	-	-	-	-	-	-	81.7	13.0
4	92	78	72	86	-	-	-	-	-	-	82.0	9.0
5	87	71	-	-	-	-	-	-	63	65	71.5	11.0
10	97	73	69	72	41	55	89	57	-	-	69.3	18.0
15	83	82	-	-	69	59	83	73	56	76	72.6	11.0
20	-	-	-	-	61	61	72	55	67	84	66.7	10.0
42	-	-	-	-	94	86	81	79	-	-	85.0	6.7
63	-	-	-	-	83	76	95	77	91	64	81.0	11.0

- = NOT DONE

s.d.= STANDARD DEVIATION.

APPENDIX TABLE 34.

MIGRATION INDICES OF LEUCOCYTES FROM SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	66	69	64	32	37	38	36	51	Mean	s.d.
- 48 hrs.	93.5	100.3	93.5	100.0	112.0	93.7	113.8	90.0	99.6	8.9
0	109.0	92.5	91.2	95.0	84.3	93.0	96.0	91.0	94.0	7.0
2	73.4	98.0	74.7	60.4	87.4	101.0	133.0	157.0	98.0	32.0
5	63.6	42.7	90.2	41.3	-	-	77.6	96.5	68.6	23.0
8	53.6	60.3	85.0	48.1	121.0	104.0	86.1	91.6	81.0	25.0
10	27.4	69.2	-	-	-	-	-	-	48.3	29.5
14	60.5	81.8	51.4	46.9	79.2	76.5	92.8	82.7	71.1	16.0
21	76.2	84.3	33.5	30.0	62.5	79.9	96.3	78.4	67.6	24.0
28	52.0	95.3	-	-	47.5	78.2	79.5	72.6	70.8	18.0
35	58.2	60.7	28.6	43.0	48.0	63.6	-	-	50.4	13.0

- : Not Done.

APPENDIX TABLE 41.

TOTAL SERUM PROTEIN CONTENTS OF SUSCEPTIBLE SHEEP - TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	37	38	36	51	66	69	32	64	Mean	s.d.
0	67	68	68	68	69	72	69	71	69.0	1.7
1	70	67	68	68	69	69	68	69	68.5	0.9
3	66	60	71	70	63	82	60	62	66.7	7.5
5	69	69	66	70	62	67	67	65	66.1	2.9
7	68	63	67	65	63	67	62	67	65.3	2.3
8	67	64	66	71	64	66	67	69	67.1	2.1
9	77	73	66	67	65	65	66	65	68.0	4.5
10	79	76	65	75	62	67	69	71	70.5	5.8
11	79	77	68	68	64	71	68	66	70.1	5.3
12	77	77	67	68	67	66	66	68	69.5	4.7
13	75	73	63	70	65	73	68	65	69.0	4.4
14	76	70	61	69	67	71	64	63	67.6	4.9
21	75	71	69	77	69	71	66	68	70.7	3.6
28	72	72	69	79	70	67	74	65	71.0	4.3
35	77	68	71	76	68	68	65	66	69.9	4.4

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 42.

ALBUMIN LEVELS* OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	34.60	31.20	35.90	28.40	31.60	34.50	33.30	34.60	33.0	2.46
1	35.00	36.10	33.30	27.80	31.60	37.40	32.40	35.00	33.6	3.01
3	28.80	29.90	27.60	27.30	27.00	41.20	29.00	28.90	30.0	4.60
5	27.70	34.30	28.40	27.30	29.00	28.70	29.40	28.00	29.1	2.21
6	29.20	27.20	30.30	26.10	28.30	30.70	28.00	30.10	28.7	1.62
7	29.10	27.30	25.20	28.30	29.00	33.20	36.20	33.70	30.3	3.70
8	31.30	32.80	29.00	28.30	29.20	32.70	35.20	31.30	31.2	2.33
9	37.50	34.20	26.10	24.80	25.80	35.40	31.20	32.80	31.0	4.85
10	39.70	34.00	31.30	32.60	28.70	35.30	26.40	31.60	32.4	4.07
11	36.90	34.50	25.30	27.30	30.70	32.40	30.90	32.70	31.3	3.73
12	30.60	39.10	23.30	28.00	24.90	39.60	29.30	30.00	30.6	5.95
13	31.30	34.90	23.90	27.00	25.60	37.90	29.40	30.10	30.0	4.69
14	36.60	35.00	27.00	29.40	27.70	32.80	31.60	23.10	31.8	3.49
21	37.40	38.20	29.80	27.70	27.90	32.20	40.80	32.40	33.3	4.95
28	29.40	33.00	29.80	27.70	27.90	32.80	37.00	34.00	31.4	3.27
35	30.60	28.50	31.80	27.30	30.00	38.20	35.90	33.60	32.0	3.71

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 43.

ALPHA$_1$ - GLOBULIN LEVELS OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12 *

Days Post Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	3.30	4.80	2.00	2.00	3.40	2.90	2.80	4.20	Mean	s.d.
1	2.80	4.00	3.40	3.40	3.40	3.50	2.70	4.80	3.50	0.66
3	3.90	4.20	6.40	3.50	3.10	3.30	3.60	3.10	3.9	1.08
5	4.90	4.10	4.60	4.20	3.10	1.90	3.80	4.60	3.9	0.98
6	3.40	5.20	3.40	3.30	3.80	3.30	4.40	4.70	3.93	0.74
7	3.40	4.40	5.30	2.80	3.20	3.30	2.70	4.10	3.65	0.89
8	2.00	5.20	4.60	3.40	3.20	2.60	5.30	3.90	3.78	1.20
9	3.80	5.10	5.20	4.50	2.50	2.00	4.20	4.30	3.95	1.15
10	4.00	4.90	4.10	2.00	2.60	3.50	3.40	2.60	3.39	0.95
11	4.70	2.10	4.70	3.40	2.70	3.30	3.30	3.40	3.45	0.89
12	4.60	5.00	4.40	3.50	3.90	2.20	4.80	3.90	4.0	0.90
13	4.50	4.30	4.30	2.80	2.70	3.60	3.80	3.10	3.64	0.71
14	4.60	4.10	3.50	1.50	2.10	4.30	3.30	4.10	3.44	1.10
21	3.00	4.40	3.50	2.40	2.80	4.00	2.20	2.60	3.11	0.78
28	3.60	4.00	3.50	2.40	2.70	4.00	2.20	2.60	3.12	0.73
35	3.10	3.40	3.50	3.00	2.70	2.70	2.60	3.30	3.04	0.35

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 44.

ALPHA$_2$ - GLOBULIN LEVELS* OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	10.70	13.60	7.40	8.80	8.20	8.60	9.70	7.80	9.35	2.01
1	11.90	10.00	8.80	8.10	8.20	8.30	8.10	6.90	8.79	1.52
3	11.10	9.50	12.00	9.80	8.80	10.70	7.90	8.00	9.73	1.47
5	13.90	11.00	9.20	7.70	6.80	8.90	9.60	7.80	9.36	2.24
6	13.60	12.30	10.10	8.50	8.80	10.00	8.70	8.00	10.00	1.99
7	14.20	10.10	13.30	9.20	7.70	9.30	8.00	13.10	10.60	2.55
8	10.70	9.70	8.60	7.40	7.10	11.80	10.60	7.20	9.14	1.82
9	12.30	11.60	11.10	12.00	7.40	10.00	9.00	7.80	10.15	1.91
10	14.30	13.60	10.90	6.10	8.90	10.60	10.10	7.90	10.30	2.74
11	13.40	10.00	8.00	8.20	7.30	8.60	9.20	8.20	9.11	1.91
12	16.10	11.50	7.60	9.80	9.80	9.50	8.90	7.20	10.05	2.79
13	14.20	12.40	12.30	9.70	9.40	10.70	8.30	6.90	10.49	2.40
14	12.20	12.60	11.10	10.00	9.70	8.50	8.60	6.80	9.94	1.98
21	12.00	9.90	11.10	11.90	8.40	9.40	10.40	9.10	10.30	1.32
28	11.50	10.80	11.00	11.90	8.40	9.40	8.60	9.10	10.10	1.37
35	13.80	10.20	11.30	12.90	9.50	8.60	8.50	9.20	10.50	1.99

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 45.

BETA-GLOBULIN LEVELS* OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	3.30	5.40	3.30	5.40	6.20	4.30	4.20	4.20	4.54	1.04
1	3.50	4.70	3.40	5.40	4.80	4.90	4.70	4.80	4.52	0.70
3	5.90	4.20	3.50	4.90	4.40	4.90	2.40	3.10	4.16	1.12
5	5.50	4.80	3.30	6.30	4.30	4.50	3.20	5.20	4.64	1.06
6	5.40	5.20	3.40	5.20	3.10	4.70	6.20	4.00	4.65	1.07
7	6.80	5.10	4.00	6.40	4.70	5.30	3.40	5.50	5.15	1.14
8	6.70	3.90	5.30	6.70	5.80	3.30	4.60	3.30	4.95	1.40
9	6.10	5.80	4.60	6.80	4.90	4.00	4.20	5.00	5.17	0.97
10	5.60	5.30	4.10	4.10	4.50	3.50	5.40	3.30	4.47	0.88
11	7.10	8.40	8.00	4.80	6.00	4.60	2.60	4.10	5.70	2.03
12	7.70	3.80	7.60	5.60	5.90	5.90	6.10	4.60	5.90	1.33
13	7.50	5.80	8.60	6.20	7.40	5.00	5.10	3.80	6.17	1.58
14	5.30	3.50	5.50	3.90	3.50	6.40	4.60	4.10	4.60	1.05
21	4.50	2.80	4.90	4.00	4.90	4.70	4.40	4.50	4.34	0.69
28	7.20	5.70	4.90	4.00	4.90	4.70	4.40	4.50	5.04	1.00
35	4.60	6.80	4.20	4.60	5.50	3.40	5.20	4.60	4.86	1.00

s.d. = standard deviation.

* = g/l.

APPENDIX TABLE 46.

GAMMAGLOBULIN LEVELS* OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	14.7	12.9	19.0	23.1	19.2	20.1	19.4	19.8	18.5	3.2
1	16.8	12.0	19.1	23.1	20.6	15.2	19.6	17.9	18.0	3.4
3	15.7	11.9	21.3	24.5	19.5	24.7	17.5	18.5	19.2	4.3
5	17.3	14.4	20.5	24.5	18.5	19.8	14.9	20.1	18.8	3.3
6	16.3	14.9	N.D.	N.D.	N.D.	N.D.	24.3	19.6	18.8	4.2
7	14.9	16.5	20.2	22.2	18.8	18.0	16.8	19.3	18.3	2.3
8	16.0	12.9	18.6	24.1	20.0	15.3	N.D.	N.D.	17.8	4.0
9	16.9	16.0	18.5	21.6	19.4	15.0	16.8	19.6	18.0	2.2
10	15.9	17.4	18.3	27.0	20.9	15.3	20.8	21.4	19.6	3.8
11	16.5	16.1	17.7	23.2	19.1	17.7	22.3	20.4	19.1	2.6
12	17.6	16.9	16.0	24.6	25.3	17.2	19.7	19.8	19.6	3.5
13	17.1	15.3	15.2	23.1	21.6	16.1	19.1	19.6	18.4	3.0
14	17.5	15.4	14.8	24.3	22.2	15.0	17.9	18.8	18.2	3.5
21	18.0	15.6	22.2	15.5	22.2	19.2	17.8	19.1	18.7	2.6
28	21.5	17.9	20.2	28.8	25.8	16.8	16.3	16.2	20.4	4.7
35	24.5	19.0	21.2	31.7	20.5	15.0	18.3	15.2	20.7	5.4

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 47.

IgG_1* LEVELS OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	8.4	3.5	10.5	11.3	6.7	15.6	17.4	16.6	11.25	5.0
1	10.6	4.6	11.3	9.0	5.2	15.0	15.7	13.5	10.6	4.2
3	10.0	4.6	10.5	9.7	7.4	15.7	16.6	12.7	10.9	4.0
5	9.5	4.6	12.8	11.3	6.7	13.0	15.7	12.7	10.8	3.7
6	9.5	4.6	N.D.	N.D.	N.D.	N.D.	15.7	12.7	10.6	4.7
7	9.5	5.1	9.7	9.7	6.7	15.7	15.7	14.3	10.8	4.0
8	6.7	7.8	9.0	10.5	9.0	15.6	15.6	12.7	10.9	3.4
9	7.3	7.3	6.7	11.3	7.4	15.7	15.7	13.5	10.6	3.9
10	8.9	5.1	7.5	11.3	9.0	16.6	16.6	12.7	11.0	4.2
11	10.6	10.0	8.2	10.5	8.0	15.7	15.7	12.7	11.4	3.0
12	5.6	7.3	8.3	11.3	9.0	15.7	15.7	12.7	10.7	3.8
13	5.1	6.7	8.2	11.3	8.2	15.6	15.7	12.7	10.4	4.0
14	7.3	8.4	8.2	11.3	6.7	16.6	15.7	12.7	10.85	3.8
21	4.6	7.8	8.2	12.8	12.8	16.6	15.6	13.5	11.5	4.2
28	8.9	8.9	8.2	10.4	11.3	15.6	13.9	13.5	11.3	2.7
35	7.8	9.0	9.0	10.5	10.5	15.9	13.9	13.5	11.3	2.85

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 48

IgG_2 LEVELS* OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	0.4	0.9	2.5	2.0	4.3	4.4	6.5	4.4	3.2	2.1
1	0.9	1.4	3.0	2.0	3.8	4.4	6.1	4.4	3.3	1.8
3	1.4	0.9	2.9	1.6	3.4	4.4	5.8	4.4	3.1	1.7
5	1.4	1.4	2.9	2.0	3.4	4.4	6.1	4.4	3.3	1.7
6	1.4	1.4	N.D.	N.D.	N.D.	N.D.	6.1	4.8	3.4	2.4
7	1.4	2.5	2.9	2.5	3.8	4.7	6.1	4.8	3.6	1.5
8	1.9	2.5	2.9	2.8	2.9	4.7	N.D.	N.D.	3.0	0.9
9	1.9	2.5	2.5	2.5	2.9	4.7	6.5	5.2	3.6	1.7
10	1.4	2.5	2.9	2.0	2.9	4.7	6.8	5.0	3.5	1.8
11	2.5	3.0	2.9	2.0	3.8	4.7	6.5	4.8	3.8	1.5
12	2.3	3.0	1.6	2.5	4.8	5.1	6.8	5.2	3.9	1.8
13	2.5	3.0	2.0	2.5	2.5	5.1	7.1	4.8	3.7	1.8
14	3.0	3.0	2.9	2.6	2.9	5.1	6.8	5.2	3.9	1.6
21	3.5	2.5	4.8	3.4	6.6	5.1	6.8	4.8	4.7	1.5
28	3.0	3.5	7.5	3.5	4.3	5.1	7.5	5.6	5.0	1.8
35	3.5	2.5	7.1	2.9	4.3	6.5	6.5	5.8	4.9	1.8

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 49.

IgM LEVELS* OF SUSCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	1.2	2.6	2.7	2.4	2.0	4.0	4.7	3.6	2.9	1.1
1	1.4	1.6	2.7	1.2	2.4	4.0	5.0	3.9	2.8	1.4
3	1.4	1.9	1.6	1.4	2.2	4.0	5.0	3.6	2.6	1.4
5	1.4	1.9	1.4	1.8	1.4	4.2	4.7	3.9	2.6	1.4
6	1.2	1.9	N.D.	N.D.	N.D.	N.D.	5.7	3.6	3.1	2.0
7	0.9	2.3	1.4	2.0	2.7	4.0	3.7	3.9	2.6	1.2
8	0.9	2.3	2.7	2.2	2.4	3.7	N.D.	N.D.	2.4	0.9
9	1.6	2.8	2.9	2.0	2.7	4.0	3.7	4.2	3.0	0.9
10	1.4	1.9	3.2	2.0	2.7	4.2	4.0	5.1	3.1	1.3
11	1.4	2.8	2.4	1.6	2.0	3.7	4.0	5.4	2.9	1.4
12	1.5	1.9	2.7	2.0	2.8	4.2	4.2	4.4	3.0	1.2
13	1.4	1.6	2.7	2.0	2.8	4.2	4.0	4.4	2.9	1.2
14	1.4	1.9	1.2	1.8	2.8	4.2	5.6	4.7	2.95	1.7
21	1.6	2.3	1.6	1.8	2.8	4.2	4.6	4.5	2.9	1.3
28	1.6	2.1	2.9	1.8	2.8	4.0	4.5	5.1	3.1	1.3
35	2.6	2.3	1.4	1.6	2.7	4.2	4.7	5.6	3.1	1.5

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 50.

ORF ANTIBODY TITRES* OF SUCEPTIBLE SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING INFECTED WITH ORF VIRUS - GROUP 12.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	37	38	36	51	66	69	32	64	Mean	s.d.
0	0	2	2	0	0	2	0	0	0.75	1.03
1	0	2	2	0	0	2	0	0	0.75	1.03
3	0	2	2	0	0	2	0	0	0.75	1.03
5	0	2	0	0	0	0	0	0	0.25	0.71
6	2	2	N.D.	N.D.	N.D.	N.D.	0	2	1.50	1.00
7	2	2	2	2	0	2	0	2	1.50	0.93
8	2	2	2	2	0	2	N.D.	N.D.	1.67	0.82
9	2	2	2	0	2	2	2	2	1.75	0.71
10	2	2	2	2	2	2	2	2	2.0	0.00
11	2	3	3	3	0	3	3	3	2.12	0.99
12	2	3	3	3	0	2	2	2	2.12	0.99
13	2	2	2	2	0	3	2	2	1.87	0.83
14	2	2	2	2	0	3	2	2	1.87	0.83
21	3	2	2	3	2	3	2	2	2.37	0.52
28	3	2	2	3	3	3	2	2	2.50	0.53
35	4	2	2	3	3	3	2	2	2.62	0.74

s.d. = standard deviation.

N.D. = Not Done.

* = Log_2

APPENDIX TABLE 51.

TOTAL SERUM PROTEIN CONTENTS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	84	71	77	71	83	70	70	66	74.0	6.6
1	73	69	73	64	73	69	69	62	69.0	4.2
2	76	79	69	66	73	65	70	62	70.0	5.8
4	80	69	65	69	70	66	63	66	68.5	5.2
6	69	64	69	67	64	64	68	63	66.0	2.5
8	74	66	64	69	71	66	64	67	67.6	3.5
9	68	67	69	71	68	67	64	67	67.6	2.0
10	71	N.D.	N.D.	69	68	67	N.D.	64	67.8	2.6
11	70	69	65	67	66	73	64	64	67.2	3.2
12	74	70	66	70	71	70	64	67	69.0	3.2
13	71	N.D.	64	72	71	70	62	69	68.4	3.9
14	71	70	70	76	71	70	65	69	70.2	3.0
21	69	64	71	69	70	69	66	69	68.4	2.3
28	71	69	67	68	64	76	67	64	68.2	3.9
35	67	66	74	63	65	66	69	63	66.6	3.6

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 52.

ALBUMIN LEVELS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	38.60	30.00	41.60	41.00	37.20	34.30	31.50	34.90	36.10	4.22
1	30.30	25.40	42.20	30.90	34.20	32.20	29.50	30.80	33.90	4.84
2	37.00	37.20	38.40	30.90	40.00	28.00	39.90	33.90	35.60	4.36
4	41.50	41.80	35.90	33.60	29.40	37.90	35.30	30.30	35.70	4.62
6	37.80	25.50	31.70	31.60	33.50	31.90	28.40	30.20	33.30	3.60
8	34.90	35.20	31.90	32.20	36.70	30.60	26.80	30.20	32.30	3.21
9	31.90	34.90	39.30	31.10	32.60	31.60	29.30	32.30	32.90	3.03
10	37.80	N.D.	N.D.	32.90	32.90	30.90	N.D.	28.30	34.60	3.48
11	37.10	32.90	35.80	34.90	29.90	36.40	29.30	34.10	33.80	2.91
12	37.10	32.20	35.20	36.40	32.10	36.40	32.50	28.90	33.80	2.87
13	34.30	N.D.	33.80	33.20	30.70	38.50	29.60	31.60	33.10	2.93
14	35.00	29.40	35.00	35.10	30.00	37.80	31.20	30.20	32.96	3.13
21	36.40	30.30	35.40	37.00	30.10	37.00	32.90	41.20	35.00	3.78
28	31.40	34.30	38.30	37.50	26.80	39.80	34.90	39.90	35.40	4.53
35	31.60	31.60	36.80	34.10	28.00	36.80	26.10	36.60	32.70	4.12

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 53.

ALPHA$_1$-GLOBULIN LEVELS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	5.00	4.30	4.60	3.50	6.60	4.90	5.60	3.30	4.72	1.08
1	3.60	4.80	3.60	3.90	6.60	4.10	6.20	3.70	4.56	1.20
2	6.00	6.30	2.10	3.90	3.60	5.90	3.50	3.70	4.37	1.51
4	6.80	3.40	0.60	3.40	5.60	2.00	2.50	5.30	3.70	2.07
6	2.70	3.80	4.10	4.00	3.90	2.50	6.80	3.80	3.95	1.30
8	3.70	4.70	4.50	3.40	2.70	4.70	7.60	6.00	4.66	1.55
9	4.10	4.00	3.40	3.50	3.40	4.00	5.10	5.40	4.11	0.76
10	3.60	N.D.	N.D.	3.40	4.10	5.40	N.D.	3.20	3.94	0.88
11	3.50	3.40	3.20	2.70	4.70	3.60	4.50	3.20	4.60	0.68
12	3.00	5.60	4.00	3.50	5.00	4.20	4.50	4.70	4.31	0.81
13	3.60	N.D.	3.80	3.60	5.00	4.90	4.90	4.10	4.27	0.61
14	3.60	4.90	4.20	3.10	5.70	4.20	5.20	4.10	4.37	0.85
21	3.40	3.90	2.80	1.40	7.00	2.10	4.60	2.10	4.41	1.75
28	5.00	3.40	3.40	2.00	6.40	1.50	4.70	1.30	4.46	1.83
35	5.40	4.60	2.90	1.30	6.50	2.60	4.80	2.50	3.83	1.76

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 54.

ALPHA$_2$-GLOBULIN LEVELS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	775	882	936	943	945	927	Mean	s.d.
0	10.90	11.40	10.00	11.30	11.60	8.40	9.80	8.60	10.25	1.26
1	10.90	11.70	8.70	9.70	9.50	6.90	11.00	8.60	9.63	1.57
2	9.10	10.30	7.50	9.90	7.30	9.10	9.80	8.60	8.95	1.10
4	9.60	8.20	7.20	9.60	8.40	5.30	8.80	9.90	9.40	1.53
6	6.90	10.20	11.00	10.10	7.10	7.00	9.50	10.10	8.99	1.70
8	7.10	8.00	9.60	9.60	6.80	8.00	8.90	9.40	9.43	1.11
9	9.50	9.40	8.30	11.30	8.20	7.40	8.90	8.70	8.96	1.16
10	9.30	N.D.	N.D.	11.00	8.00	6.70	N.D.	9.70	8.94	1.65
11	8.40	8.90	7.80	8.10	8.60	8.00	9.50	9.00	9.54	0.58
12	9.60	11.20	8.60	11.20	9.30	7.00	7.60	10.80	9.41	1.61
13	10.00	N.D.	8.30	10.80	10.70	9.10	7.40	10.30	9.51	1.29
14	9.30	9.80	9.80	10.70	9.10	9.10	8.50	9.60	9.49	0.65
21	8.90	9.00	8.50	11.70	9.60	8.20	8.60	8.20	9.09	1.15
28	11.40	9.60	8.10	10.20	9.60	7.60	8.70	7.70	9.11	1.33
35	8.70	9.90	11.00	11.40	9.10	7.20	11.70	8.80	9.73	1.56

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 55.

BETA-GLOBULIN LEVELS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	7.60	5.70	3.90	4.90	4.10	4.90	6.30	2.60	5.00	1.55
1	5.80	4.80	5.10	5.80	3.60	4.80	6.90	4.90	5.20	0.97
2	6.80	6.30	4.80	3.90	2.90	5.20	4.20	1.20	5.41	1.81
4	7.20	2.70	4.60	4.10	4.90	4.00	5.00	3.80	4.54	1.30
6	5.50	11.40	6.20	4.00	2.60	3.20	6.80	2.70	5.30	2.94
8	6.70	5.30	5.10	4.80	3.40	3.30	5.10	4.00	4.71	1.12
9	6.10	5.40	4.80	4.90	5.40	4.70	5.70	3.20	5.02	0.88
10	5.00	N.D.	N.D.	5.50	4.10	5.40	N.D.	3.90	4.78	0.74
11	4.90	6.90	6.50	4.00	4.00	4.40	5.10	3.40	4.90	1.24
12	6.70	7.00	6.70	8.40	3.60	4.90	4.50	4.10	5.74	1.69
13	5.00	N.D.	5.10	5.00	2.90	4.90	4.90	3.40	4.46	0.91
14	5.70	8.40	6.30	7.60	4.30	5.60	4.60	4.10	5.83	1.55
21	5.50	4.50	7.10	4.10	3.50	4.80	5.40	2.70	4.70	1.35
28	7.80	6.90	4.00	3.40	4.50	4.50	4.70	2.60	4.80	1.73
35	6.00	5.90	5.90	3.20	3.90	4.60	5.50	2.50	4.69	1.36

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 56.

GAMMAGLOBULIN LEVELS OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	21.8	20.0	16.9	19.1	23.1	17.5	16.8	16.5	19.0	2.5
1	21.8	22.0	13.1	14.2	18.9	20.6	15.1	13.6	17.4	3.8
2	16.6	19.0	15.8	17.1	18.9	16.9	12.6	14.2	16.4	2.2
4	16.9	12.3	17.0	17.8	18.2	17.3	11.4	17.1	16.0	2.6
6	15.8	14.7	15.8	17.8	17.4	19.1	16.2	16.4	16.7	1.4
8	18.6	13.3	12.7	18.5	18.3	20.0	15.3	17.5	16.8	2.7
9	17.7	13.4	13.1	19.8	18.3	19.5	14.7	17.5	16.8	2.7
10	15.7	13.0	N.D.	15.8	19.2	18.8	N.D.	18.0	16.8	2.4
11	16.1	16.5	11.7	17.5	20.0	20.4	15.3	14.2	16.5	2.9
12	17.8	14.0	12.0	17.5	21.4	17.5	14.7	18.8	16.7	3.0
13	18.6	15.0	12.7	18.7	20.7	12.6	14.8	19.3	16.6	3.2
14	17.9	17.5	14.7	22.1	22.1	16.1	15.6	20.6	18.3	2.9
21	18.4	16.7	17.0	19.4	18.9	16.5	14.5	19.4	17.6	1.7
28	18.7	14.4	18.4	15.0	16.6	18.1	14.1	19.2	16.8	2.1
35	15.5	14.5	16.9	13.3	N.D.	16.5	N.D.	19.6	16.9	2.1

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 57.

IgG_1* LEVELS OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	11.7	12.3	13.8	11.6	13.0	12.0	11.8	12.4	12.3	0.7
1	7.6	9.6	13.6	12.7	8.4	9.9	12.0	12.4	10.8	2.2
2	7.6	10.8	9.0	8.5	10.6	10.9	11.4	11.8	10.1	1.5
4	6.7	7.3	7.9	13.9	10.6	7.9	11.4	8.6	9.3	2.5
6	11.7	10.8	13.1	13.8	9.5	6.9	12.3	12.1	10.5	2.2
8	12.8	10.3	10.1	7.5	8.4	10.9	12.0	11.9	10.5	1.8
9	13.4	9.6	12.2	10.5	12.7	10.2	12.1	12.4	11.0	1.3
10	13.9	8.5	N.D.	12.7	8.4	8.9	N.D.	11.2	10.6	2.4
11	8.6	8.5	14.6	10.5	7.3	10.3	12.1	11.8	10.5	2.4
12	7.3	8.5	13.1	11.2	16.9	8.5	9.0	11.2	10.7	3.1
13	13.1	9.0	12.4	11.5	15.6	10.2	12.4	12.5	12.1	2.0
14	7.3	13.0	14.9	11.6	14.5	10.8	12.9	12.1	12.1	2.4
21	14.7	13.4	12.9	11.61	13.1	13.6	12.1	11.8	12.9	1.0
28	16.3	12.9	14.9	11.7	14.4	13.6	9.0	12.1	13.1	2.2
35	8.0	12.9	14.7	10.5	13.1	13.9	12.6	12.2	12.3	2.1

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 58.

IgG_2 LEVELS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	1.4	3.5	5.0	6.3	1.7	2.2	3.8	1.6	3.2	1.8
1	1.4	2.7	5.6	7.2	1.7	2.2	4.3	2.0	3.4	2.1
2	1.4	3.7	5.0	8.2	1.7	1.8	3.2	1.6	3.4	2.4
4	1.4	3.1	5.6	7.9	2.2	2.2	4.3	2.4	3.6	2.2
6	1.9	3.1	7.7	9.7	2.2	1.8	4.3	2.4	4.1	3.0
8	1.9	3.5	5.6	8.7	3.3	1.5	4.3	2.0	3.8	2.4
9	2.3	3.1	6.3	8.7	4.4	1.8	4.8	1.6	4.1	2.5
10	1.4	3.1	N.D.	8.2	3.3	1.5	N.D.	1.6	3.2	2.6
11	1.4	3.5	6.3	8.7	2.7	1.8	3.7	2.4	3.8	2.5
12	1.9	3.4	7.7	7.7	2.7	1.8	4.8	2.0	4.0	2.5
13	1.9	3.5	6.3	9.2	2.2	2.2	5.3	2.0	4.1	2.6
14	1.4	3.9	6.0	9.2	1.7	2.2	5.0	2.0	3.9	2.7
21	1.8	3.9	5.9	7.7	3.3	2.6	3.8	2.0	3.9	2.0
28	1.8	3.1	6.3	9.7	3.3	2.2	3.8	2.0	4.0	2.4
35	1.8	2.4	6.4	8.7	3.3	3.1	2.4	2.0	3.8	2.5

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 59.

IgM LEVELS* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	6.5	5.9	4.3	3.2	3.9	3.1	4.2	2.1	4.2	1.5
1	5.6	4.4	3.8	3.2	3.3	3.1	3.3	2.4	3.6	1.0
2	4.2	5.9	3.0	3.2	3.9	2.7	3.3	3.8	3.6	1.1
4	4.7	3.9	3.0	5.2	3.9	2.3	3.3	2.1	3.6	1.1
6	4.7	5.4	3.8	4.6	2.9	2.9	4.4	2.4	3.8	1.2
8	5.6	6.5	3.4	6.7	4.9	2.7	3.8	3.5	4.3	1.5
9	6.1	5.9	4.7	2.6	3.9	2.3	3.8	2.9	4.3	1.9
10	6.0	4.7	N.D.	3.9	3.9	2.3	N.D.	4.3	4.2	1.2
11	5.6	6.4	5.2	4.6	3.4	1.9	3.3	4.7	4.4	1.4
12	4.2	7.0	4.2	3.9	3.9	2.7	2.5	5.2	4.2	1.4
13	4.2	4.5	3.8	3.9	3.4	2.3	2.9	3.9	3.6	0.7
14	4.2	4.7	3.8	4.6	2.9	2.7	3.8	3.9	3.8	0.7
21	4.2	5.4	5.6	4.6	3.4	3.5	6.7	2.7	4.5	1.3
28	3.8	3.9	3.4	5.2	3.4	3.5	5.3	3.1	3.9	0.8
35	3.1	3.0	3.8	3.2	2.4	3.5	5.2	4.3	3.6	0.9

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 60.

ORF ANTIBODY TITRES* OF PREVIOUSLY INFECTED SHEEP TREATED WITH CORTICOSTEROID BEFORE BEING CHALLENGED WITH ORF VIRUS - GROUP 13

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	836	775	777	882	936	943	945	927	Mean	s.d.
0	2	0	0	0	0	2	2	0	0.75	1.03
1	2	0	0	0	0	2	2	0	0.75	1.03
2	2	0	0	0	0	2	2	2	1.25	1.03
4	2	0	0	2	0	0	2	2	1.00	1.07
6	2	0	0	2	0	2	2	3	1.37	1.19
8	2	0	0	2	0	2	2	3	1.37	1.19
9	2	0	2	2	2	2	2	3	1.87	0.83
10	2	N.D.	N.D.	3	2	2	N.D.	4	2.12	1.12
11	2	0	2	3	2	2	2	4	2.12	1.12
12	3	0	2	3	3	2	2	N.D.	2.14	1.07
13	3	2	2	3	3	2	2	4	2.62	0.74
14	3	2	2	3	3	2	3	3	2.62	0.52
21	3	2	2	3	3	3	4	3	2.87	0.64
28	2	2	2	3	4	3	4	3	2.87	0.83
35	2	3	2	3	4	2	5	4	3.12	1.12

s.d. = standard deviation.

N.D. = Not Done.

* = Log_2.

APPENDIX TABLE 61.

TOTAL SERUM PROTEIN CONTENTS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	77	74	76	63	76	67	76	64	71.6	5.93
1	84	75	80	63	71	70	73	67	72.9	6.79
2	80	71	76	64	76	71	76	70	73.0	4.99
4	81	71	71	69	74	67	70	66	71.1	4.70
6	78	74	70	66	74	65	68	67	70.2	4.6
8	74	74	73	71	66	66	66	66	69.5	3.85
9	77	80	72	69	70	69	71	67	71.9	4.42
10	77	N.D.	N.D.	69	69	67	N.D.	64	69.2	4.82
11	70	76	73	67	70	71	66	66	69.9	3.52
12	80	78	72	70	78	69	70	69	73.3	4.62
13	73	N.D.	71	71	71	70	65	64	69.3	3.40
14	73	81	74	70	71	73	73	69	73.0	3.66
21	71	76	71	70	67	69	69	67	70.0	2.88
28	69	74	72	69	70	69	70	68	70.1	1.96
35	74	74	78	67	70	66	D	63	70.3	5.31

s.d. = standard deviation.

D = Died.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 62.

ALBUMIN LEVELS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	48.50	37.50	32.50	22.10	30.20	38.30	34.00	31.50	34.30	7.60
1	47.00	39.70	36.70	27.10	27.80	37.80	33.50	36.30	35.70	6.44
2	40.70	32.10	36.30	21.90	37.80	38.60	33.30	37.80	34.80	5.92
4	39.00	40.00	35.50	24.70	31.90	45.70	32.80	38.80	36.00	6.34
6	36.80	38.20	30.10	27.60	31.90	29.90	25.70	30.90	31.40	4.26
8	33.40	33.10	31.30	31.10	23.00	35.90	26.60	30.30	30.59	4.08
9	34.70	37.00	31.70	24.70	29.40	36.40	26.90	37.60	32.30	4.90
10	39.30	N.D.	N.D.	24.70	30.90	32.90	N.D.	38.00	33.16	5.87
11	35.70	41.2	34.20	26.90	30.10	40.00	28.60	39.50	34.50	5.52
12	36.70	35.00	33.90	23.10	31.10	35.70	35.00	37.00	33.44	4.56
13	32.80	N.D.	30.40	24.00	29.30	23.80	27.30	35.40	29.00	4.33
14	34.90	42.2	33.40	21.70	35.70	29.10	29.80	35.70	32.80	6.05
21	32.80	42.30	35.40	28.00	28.20	34.30	27.40	40.30	33.60	5.66
28	30.20	40.10	42.50	29.80	26.60	39.10	26.60	40.90	34.50	6.79
35	30.10	39.30	31.90	25.70	27.30	37.90	D.	38.10	32.90	5.55

s.d. = standard deviation.

D = Died

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 63.

ALPHA$_1$-GLOBULIN LEVELS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	3.0	3.70	3.80	3.20	3.80	3.40	3.00	3.20	3.39	0.34
1	1.70	5.20	3.20	4.40	3.60	4.20	2.90	2.00	3.40	1.20
2	4.00	3.60	3.80	4.50	1.50	6.40	6.00	3.50	4.16	1.54
4	3.20	4.30	2.10	4.80	4.50	1.30	4.90	3.30	3.55	1.32
6	3.90	4.40	4.20	3.90	3.70	5.20	3.40	4.70	4.17	0.58
8	2.20	5.90	3.60	4.20	4.60	4.70	2.70	3.90	3.975	1.17
9	3.10	5.60	2.90	3.40	4.90	3.40	4.20	3.40	3.86	0.95
10	1.50	N.D.	N.D.	2.70	3.40	5.40	N.D.	3.20	3.24	1.41
11	2.10	3.80	2.90	2.70	3.50	5.00	3.30	2.60	3.24	0.89
12	3.20	5.40	3.60	3.50	4.70	4.80	1.40	3.40	3.75	1.24
13	2.20	N.D.	4.20	4.90	4.30	6.30	2.60	2.60	3.87	1.49
14	2.90	5.70	3.70	4.90	2.90	5.80	5.10	2.10	4.14	1.42
21	2.90	3.00	3.50	2.10	3.40	4.80	4.10	1.30	3.14	1.10
28	3.40	3.70	3.60	2.10	4.20	2.70	4.20	2.70	3.32	0.76
35	5.10	3.70	2.30	2.70	7.00	3.30	D	2.50	2.8	1.70

s.d. = standard deviation.

N.D. = Not Done.

D = Died.

* = g/l.

APPENDIX TABLE 64.

$ALPHA_2$ - GLOBULIN LEVELS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	5.40	10.30	9.80	12.60	12.90	8.80	9.80	10.30	9.99	2.33
1	8.40	9.70	10.40	11.30	9.30	9.80	9.50	9.40	9.93	0.85
2	8.80	10.70	9.80	9.00	8.30	9.30	12.90	10.50	9.91	1.46
4	8.90	8.60	8.50	10.30	11.90	6.00	9.80	8.60	9.07	1.70
6	9.40	9.60	8.40	9.20	8.90	9.80	8.10	11.40	9.35	1.01
8	7.40	11.80	8.70	9.20	10.50	8.00	8.00	10.50	9.26	1.53
9	8.50	12.90	10.10	11.00	9.10	9.60	9.20	9.40	9.97	1.40
10	7.70	N.D.	N.D.	11.70	9.60	8.10	N.D.	9.00	9.22	1.57
11	7.00	9.90	6.50	8.10	9.80	7.10	8.60	8.60	8.20	1.27
12	8.80	11.70	7.90	9.10	11.70	8.90	5.60	11.70	9.42	2.18
13	10.20	N.D.	9.20	8.50	9.30	10.50	7.20	9.70	9.23	1.11
14	7.30	11.40	9.60	9.10	8.60	9.50	8.70	11.70	9.49	1.46
21	8.60	10.60	7.90	11.90	9.40	8.20	8.20	9.40	9.27	1.38
28	9.60	9.60	5.80	9.00	9.10	7.50	8.40	10.20	8.65	1.42
35	10.30	10.40	11.70	8.10	11.20	8.00	D	9.40	9.87	1.44

s.d. = standard deviation.

N.D. = Not Done.

D = Died.

* = g/l.

APPENDIX TABLE 65.

BETA-GLOBULIN LEVELS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	3.10	7.40	7.60	6.30	5.30	3.40	4.50	3.90	5.19	1.76
1	3.40	5.20	6.40	3.20	7.90	4.20	5.10	2.00	4.68	1.89
2	6.80	8.60	3.80	5.20	6.80	4.30	4.50	3.50	5.44	1.79
4	7.20	6.40	3.50	4.10	8.90	2.70	5.60	2.00	5.05	2.38
6	5.50	6.60	8.40	7.20	7.40	5.90	4.70	4.00	6.21	1.47
8	6.70	6.60	8.70	7.80	7.90	3.30	4.00	4.60	6.20	2.00
9	6.10	8.10	6.50	4.10	7.00	4.10	6.40	3.40	5.70	1.65
10	5.00	N.D.	N.D.	3.40	5.50	4.70	N.D.	2.60	4.24	1.20
11	4.90	6.10	5.80	4.70	5.60	4.30	4.00	2.60	4.75	1.14
12	6.70	7.00	6.50	5.60	6.20	4.10	3.50	2.70	5.29	1.63
13	5.00	N.D.	5.70	5.70	5.70	6.30	4.60	2.60	5.09	1.23
14	5.70	5.70	5.20	5.60	4.30	5.80	7.30	2.70	5.29	1.33
21	5.50	6.00	5.70	4.90	6.00	8.90	6.20	4.70	5.99	1.29
28	7.80	5.90	4.30	4.90	6.30	5.50	9.10	2.70	5.81	2.0
35	6.00	5.90	7.80	5.40	3.50	6.00	D	2.50	5.30	1.76

s.d. = standard deviation.

N.D. = Not Done.

D = Died.

* = g/l.

APPENDIX TABLE 66.

GAMMAGLOBULIN LEVELS OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.*

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	20.0	14.7	21.9	18.9	21.2	13.4	24.2	15.5	18.7	3.8
1	23.5	15.0	23.1	17.0	20.0	14.0	21.8	14.8	18.7	3.9
2	23.9	16.4	22.7	23.8	21.2	12.9	18.0	14.7	19.2	4.3
4	24.4	12.1	21.3	24.7	19.3	11.4	16.8	13.2	17.9	5.4
6	21.2	14.7	18.9	17.8	22.3	14.3	25.6	16.1	18.9	4.0
8	25.2	16.9	20.4	18.4	19.7	14.6	25.3	16.4	19.6	3.9
9	24.6	16.9	20.9	25.4	19.6	15.1	24.0	13.5	20.0	4.5
10	23.9	17.0	N.D.	26.1	19.2	16.1	N.D.	11.6	19.0	5.3
11	21.7	15.3	23.3	24.9	21.0	15.0	21.9	12.5	19.5	4.5
12	24.7	18.6	20.2	28.7	24.1	15.1	24.5	13.7	21.2	5.2
13	22.6	18.0	21.2	27.6	22.8	23.1	23.4	14.2	21.6	4.0
14	22.6	16.2	22.4	28.7	20.0	21.8	21.8	16.5	21.3	3.9
21	21.4	13.6	18.4	23.1	20.2	12.3	22.6	11.4	19.9	4.8
28	19.9	14.8	16.3	23.6	23.8	13.7	21.7	11.6	18.3	4.7
35	22.1	22.3	24.1	25.7	21.0	16.6	N.D.	1.00	20.3	5.3

s.d. = standard deviation.

N.D. = Not Done.

* = g/l.

APPENDIX TABLE 67.

IgG_1 LEVELS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	980	772	378	937	942	944	920	Mean	s.d.
0	14.4	10.8	10.1	13.9	15.5	7.9	14.1	9.9	12.1	2.7
1	12.8	8.5	10.4	13.1	9.5	10.9	13.9	11.1	11.3	1.9
2	16.3	7.3	11.2	10.5	10.6	6.9	9.0	12.5	10.5	3.0
4	14.3	9.6	10.8	10.7	13.0	6.9	13.2	7.4	10.7	2.7
6	13.7	7.3	11.7	11.8	10.6	6.0	14.9	15.2	11.4	3.4
8	13.4	10.8	12.3	12.8	11.8	7.9	13.2	14.8	12.1	2.1
9	14.9	9.6	13.5	18.8	14.5	6.9	15.8	13.8	13.5	3.7
10	14.7	6.3	N.D.	20.1	16.9	6.9	N.D.	14.8	13.3	5.5
11	14.7	14.7	14.6	14.8	16.3	6.9	20.0	14.8	14.6	3.6
12	14.5	14.7	13.6	14.1	13.3	6.9	21.3	11.5	13.7	4.0
13	14.0	14.2	13.8	14.8	14.8	8.5	10.8	12.5	12.9	2.2
14	14.7	14.0	13.0	15.0	14.7	12.0	11.6	12.4	13.4	1.3
21	14.8	13.2	17.0	14.9	14.4	12.0	10.0	10.4	13.3	2.4
28	14.5	13.0	11.6	15	14.8	12.0	14.0	10.5	13.2	1.7
35	14.3	13.4	14.9	15.2	13.1	13.8	D	10.5	13.3	1.7

s.d. = standard deviation.

N.D. = Not Done.

D. = Died.

* = g/l.

APPENDIX TABLE 68.

IgG_2 LEVELS OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	5.0	6.1	8.4	7.2	1.3	2.6	3.8	6.8	5.1	2.4
1	3.3	5.2	6.3	7.2	0.4	2.2	3.3	6.3	4.3	2.3
2	3.7	3.1	4.3	6.3	0.8	2.2	2.8	9.3	4.1	2.6
4	3.3	5.2	5.6	7.2	1.3	1.8	2.8	5.7	4.1	2.1
6	4.4	5.6	6.3	11.4	1.3	1.8	3.8	8.7	5.4	3.4
8	3.9	6.1	5.6	8.6	2.7	2.2	3.3	8.7	5.1	2.5
9	5.6	6.1	7.0	5.8	3.3	2.6	3.8	7.7	5.2	1.8
10	4.4	5.6	N.D.	4.6	2.7	2.2.	N.D.	7.4	4.5	1.9
11	3.9	6.1	8.4	7.7	2.7	1.8	3.8	6.3	5.1	2.4
12	3.3	6.1	7.0	6.7	3.3	2.2	2.4	6.3	4.7	2.0
13	2.8	7.9	5.6	7.7	2.7	2.2	2.4	8.7	5.0	2.8
14	2.8	11.5	5.6	8.7	3.3	2.6	3.3	9.3	5.9	3.5
21	4.4	7.9	5.6	8.2	4.4	3.5	3.3	9.3	5.8	2.3
28	4.4	5.6	6.3	7.7	4.4	4.0	2.4	9.3	5.5	2.2
35	4.4	5.2	5.0	7.7	4.4	2.6	D	9.3	5.4	2.1

s.d. = standard deviation.

N.D. = Not Done.

D = Died.

* = g/l.

APPENDIX TABLE 69.

IgM LEVELS* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	780	772	378	937	942	944	920	Mean	s.d.
0	6.0	5.6	4.7	3.2	4.9	3.1	4.2	3.1	4.3	1.1
1	6.0	4.9	5.2	3.2	2.9	3.1	5.2	3.1	4.2	1.2
2	6.0	4.4	4.3	2.0	3.4	2.7	4.2	2.4	3.7	1.3
4	5.6	4.4	5.2	3.2	3.9	2.3	3.3	3.9	4.0	1.1
6	5.6	5.4	6.6	3.9	3.4	2.3	4.2	3.9	4.4	1.4
8	5.6	4.9	4.7	3.9	4.4	2.3	4.7	4.8	4.4	1.0
9	6.5	3.9	5.2	3.2	4.9	2.7	4.7	3.1	4.3	1.3
10	6.0	4.4	N.D.	3.9	3.9	3.6	N.D.	3.5	4.2	0.9
11	4.2	5.4	5.2	3.9	2.9	3.6	4.7	3.9	4.2	0.9
12	4.7	4.9	4.3	3.9	2.9	3.6	6.7	3.9	4.4	1.1
13	4.2	5.0	5.2	3.2	2.9	3.6	3.3	3.9	3.9	0.8
14	4.7	5.4	5.2	5.2	2.4	4.0	4.2	3.9	4.4	1.0
21	4.7	5.4	5.2	2.6	2.9	3.6	5.2	3.5	4.2	1.2
28	3.8	5.4	5.2	6.7	2.9	2.7	4.7	3.9	4.4	1.3
35	4.7	4.4	4.7	4.6	2.9	4.0	D	3.9	4.1	0.6

s.d. = standard deviation.

N.D. = Not Done.

D = Died.

* = g/l.

APPENDIX TABLE 70.

ORF ANTIBODY TITRES* OF PREVIOUSLY INFECTED SHEEP CHALLENGED WITH ORF VIRUS - GROUP 14.

Days Post-Challenge	NUMBER OF SHEEP								STATISTICS	
	833	378	780	772	937	942	944	920	Mean	s.d.
0	3	2	2	3	0	2	0	2	1.75	1.16
1	3	2	2	3	0	2	0	2	1.75	1.16
2	3	2	2	3	0	2	0	2	1.75	1.16
4	3	2	2	3	2	2	2	2	2.25	0.46
6	3	3	2	4	2	2	2	3	2.62	0.74
8	4	3	3	4	3	2	2	3	3.0	0.76
9	4	3	3	4	3	2	3	3	3.12	0.64
10	4	3	3	4	3	3	3	3	3.25	0.46
11	5	3	4	4	4	3	3	3	3.62	0.74
12	5	3	4	4	4	3	3	3	3.62	0.74
13	5	3	4	5	4	3	3	3	3.75	0.89
14	5	4	4	5	4	3	4	4	4.12	0.64
21	5	4	4	5	4	3	4	4	4.12	0.64
28	5	4	4	5	4	3	4	4	4.12	0.64
35	4	3	5	4	3	3	D	4	3.62	0.74

s.d. = standard deviation.

D = Died

N.D. = Not Done.

* = Log_2